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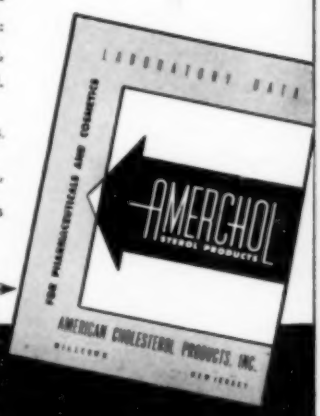

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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

JUSTIN L. POWERS, EDITOR, WASHINGTON, D. C.

VOLUME XXXVII — *OCTOBER, 1948* — NUMBER 10 — CONSECUTIVE NO. 20

The Antihistaminic Drugs*

A Review of the Literature

By THOMAS J. HALEY

THE WIDE interest in the clinical application of drugs which have a blocking action where histamine is concerned has led to many editorials (1–6) and reviews (7–23). These reports have been principally concerned with the older French antihistamine drugs and with two drugs (Benadryl and Pyribenzamine) developed and used in this country. At the present time many other drugs with antihistaminic activity are being synthesized, pharmacologically tested, and therapeutically used in allergic conditions.

Histamine itself has some definite pharmacological effects, some of which are direct and others which are indirect. Table I lists the principal actions of histamine (24).

Dragstedt (25, 26) has pointed out the significance of histamine and/or H-substances liberated in anaphylaxis and a variety of pathological conditions (Futterrehe, allergy, gastroduodenal ulcer). It is also significant that he pointed out the antihistaminic action of certain amino acids and suggested that more effective histamine blocking agents could probably be synthesized. Dragstedt compared the physiological effects of histamine with the symptoms observed in asthma, anaphylaxis, and other allergic and pathological conditions and reached the conclusion that although histamine may not always be the main causative agent of these symptoms any prevention of its effects would be of great benefit.

TABLE I.—SUMMARY OF PRINCIPAL ACTIONS OF HISTAMINE

Site of Action	Effect Produced	Some Important Areas Affected
Smooth muscle	Contraction	Bronchiolar, vascular, intestinal, and uterine smooth muscle
Capillaries	Dilatation and increased permeability	Skin and mucous membranes
Glands of external secretion	Secretogogue	Lacrimal, nasal, pulmonary, and digestive glands
Cutaneous endings of pain response	Pain	Skin

This review will attempt to present the available literature of not only those antihistaminic drugs which are in wide use but also those which are just becoming available. A comparison will be made of their chemical structure, pharmacologic action, and clinical usefulness.

* Received March 26, 1948, from the School of Medicine, University of California at Los Angeles, Calif.

CHEMISTRY

Synthesis.—Fourneau and Bovet (27) in 1933 reported the first synthesis of a successful antihistaminic agent, a phenolic ether of the ethanolamine series called 929 F.

Fleming and Rieveschl (33) have reported the synthesis of Benadryl containing radiocarbon (C_{14}). Starting with phenyl magnesium bromide and $C_{14}O_2$ and passing through five intermediates a 70% yield of Benadryl was obtained. This is reduced to a 55% over-all yield when based upon the barium carbonate used to obtain the radiocarbon.

The synthesis of Pyribenzamine, Neoantergan, and a large number of compounds of the ethylenediamine series was reported in 1946 by Huttrer, *et al.* (28). They used three different methods: (*a*) Condensation of the dialkylaminoethyl substituted amino heterocyclic compounds with an alkyl or aralkyl halide (yield 21% to 87%); (*b*) condensation of the halogenated heterocyclic substance with an asymmetrically tri-substituted alkylenediamine (yield 15% to 66%); (*c*) condensation of an alkyl or aralkyl substituted amino heterocyclic derivative with a dialkylaminoethyl halide (yield 78% to 89%). Sodamide or lithium amide was used as the condensing agent.

Clapp, *et al.* (29) have reported on the synthesis of two new antihistamines, N-(2-pyridyl)-N-(5-bromo-2-thenyl)-N-dimethylethylenediamine and its 5-chloro-2-thenyl analog. They condensed 5-bromo-2-thenyl chloride and 5-chloro-2-thenyl chloride with N-(2-pyridyl)-N-,N-dimethylethylenediamine in the presence of either sodium or potassium amide to form the antihistamines. The former was obtained in 54% and the latter in 62% yield. Weston (30) synthesized N-(2-pyridyl)-N-(2-thenyl)-N,N-dimethylethylenediamine by the condensation of N-(2-pyridyl)-N,N-dimethylethylenediamine with 2-thenyl chloride in the presence of sodamide or lithium amide, obtaining a 64% yield.

Burtner and Cusic (31) synthesized diethylaminoethyl-9,10-dihydroanthracene-9-carboxylate and diethylaminoethyl-xanthene carboxylate by interaction of the corresponding acids with diethylaminoethyl chloride in isopropanol solution. Previously they reported the synthesis of diethylaminoethyl fluorene-9-carboxylate (32).

Qualitative Analysis.—Specifications for Pyribenzamine have been reported in New and Nonofficial Remedies (34). This compound gives definite reactions with Reinecke's salt and sulfuric acid. The latter color test enables Pyribenzamine to be differentiated from Benadryl. Identifying tests for Benadryl are also reported in New and Nonofficial Remedies, 1947 (35).

Keenan (36) has shown that both Benadryl and Pyribenzamine may be identified and differentiated by their reaction with chloroplatinic acid. Haley (37) has found that these compounds can be differentiated and identified by their reactions with Mandelin's, Mecke's, Marquis', and Förhde's reagents. Identifying derivatives and the physical constants for several antihistamines are given in Table II.

Quantitative Analysis.—All of the antihistamines which contain nitrogen in their molecules may be analyzed by the Dumas or Kjeldahl methods. Also the bases may be titrated with standard acid and the acid salts with standard alkali but these methods are nonspecific. Pyribenzamine has been analyzed gravimetrically as the dipicrate (34). Benadryl has been analyzed by titration of the free base. Gelvin and McGavack (38) determined Benadryl colorimetrically in blood and spinal fluid using a modification of the Brodie and Undenfriend (39) method for organic bases.

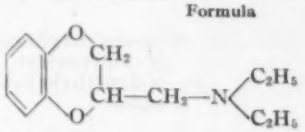
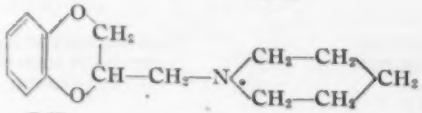
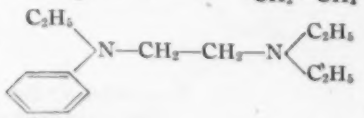
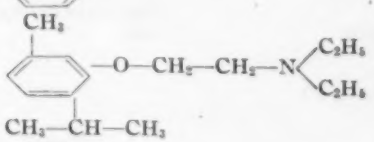
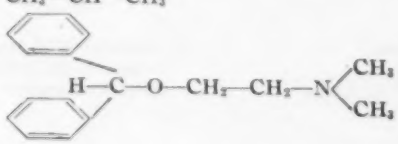
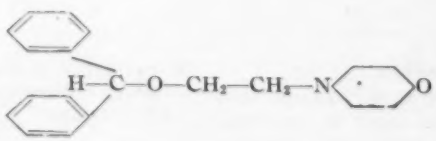
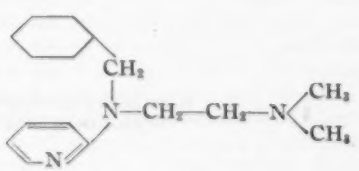
The newer compounds containing sulfur or chlorine may be analyzed after combustion and either of these elements determined by standard methods.

Effect of Chemical Structure on Activity.—When one considers the number of potent antihistamine drugs being used, several facts concerning their structure become evident. The more potent compounds contain as a basic unit either ethanolamine (Benadryl) or ethylenediamine (Pyribenzamine). Further, the terminal N atom should be a tertiary amine, otherwise the potency is of a low order (56). Also it appears that the dimethyl amine derivatives are much less toxic than those containing the diethyl grouping (i.e. Antergan and Benadryl *versus* the Fourneau compounds). However, the terminal N atom may be part of a heterocyclic compound (i.e. Antistine or the morpholine derivative of Benadryl) and have a high antihistaminic value. The chain length between the O and N or the N and N atoms should be not more than 2 C atoms because increased length as well as branching leads to less activity. However, the new phenothiazine compounds do not follow this pattern because one of the most potent compounds, 3277RP, has an isopropyl group connecting the two N atoms, but the cyclic nature of the phenothiazine molecule may be the reason for the activity of 3277RP (47). The change of the aromatic nuclei attached to the alpha N or the ether linkage tends to decrease the activity of the compound (i.e., Benadryl *versus* thymoxyethyldiethylamine or Pyribenzamine *versus* N′-pyridyl-N′-pyridyl-N-dimethylethylenediamine). However, the change from the pyridyl to the pyrimidyl group still leaves the compound with a fair degree of antihistaminic activity. Further, in these same compounds if a para-methoxyl group is added to the benzyl group an increase in antihistaminic activity occurs (Neoantergan or Neoheteramine). Changing the benzyl group to a thenyl or halogenated thenyl group leaves the compound with its original activity (45, 54). Conversion of the compounds related to Benadryl into quaternary ammonium derivatives greatly increases their activity in counteracting acetylcholine but this is to be expected because this structural change makes the compounds more closely resemble choline (52). Similarly the esters studied by Lehmann and Young (44) have a potent acetylcholine blocking action due to their resemblance to the antispasmodic drugs (i.e., Trasentin). Complete evaluation of the structure of the phenothiazine derivatives (47) and of Thephorin (51) in relation to their activity must wait until more information is available regarding structurally related compounds. Another compound, β-pyridylethylmethylamine (PT-9), which has shown great activity in combatting acute histamine cephalalgia may point the way to new compounds which will be specific against this condition as well as migraine (53). A list of some of the more potent antihistaminic drugs is given in Table III.

TABLE II.—DERIVATIVES AND PHYSICAL CONSTANTS OF SEVERAL ANTIHISTAMINES

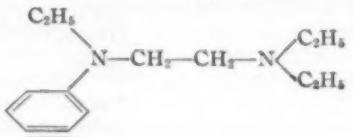
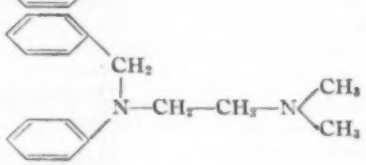
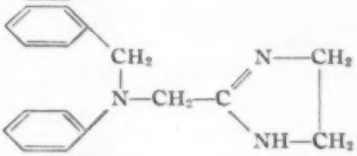
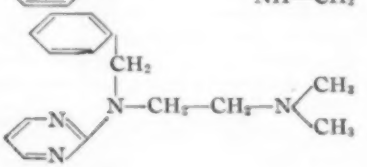
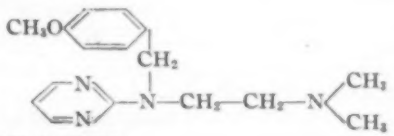
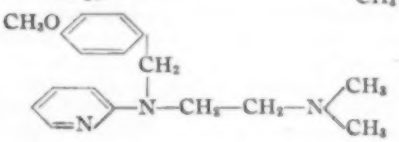
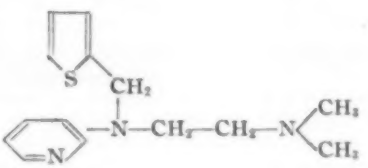
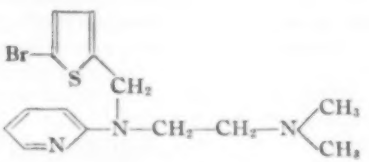
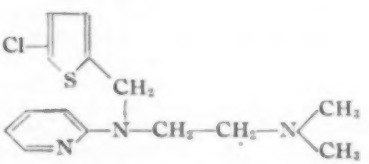
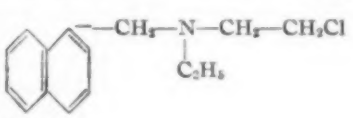
Compound	Derivative	B. P. °C	B. P. Mm.	M. P. °C.	Refractive Index	Reference
Benadryl	HCl	...	...	166–170	...	35
	Di-HCl	...	...	65	...	35
Pyribenzamine	Base	142	0.07	...	1.5759 1.5765	28
	HCl	138	...	193	...	28
	Dipicrate	...	...	185–190	...	34
Histadyl		...	...	161–162	...	..
Thenylene	HCl	...	...	159–161	...	30
Lilly 01013	Base	173–75	3	...	1.5835	30
	Methiodide	...	...	156–157 d.	...	30
Neoantergan	Base	165–173	0.07	...	1.5750	28
	HCl	...	...	143–143.5	...	28
	Maleate	...	...	100–101	...	98
Diethylaminoethyl-9,10-dihydro-anthracene carboxylate	HCl	...	...	170–171	...	31
Diethylaminoethyl-9, xanthene carboxylate	HCl	...	...	159–160	...	31
Thephorin	Tartrate	...	...	165–167	...	51
Chlorothen	Base	155–175	1	...	...	29
	HCl	...	...	106–108	...	29
Bromothen	Base	173–175	1	...	...	29
	HCl	...	...	124–126	...	29

TABLE III.—CHEMICAL STRUCTURE OF SOME ANTIHISTAMINES

Common Name	Chemical Name	Formula
883 F	Diethylamino-methyl-2-benzodioxane	
933 F	Piperidino-methyl-2-benzodioxane	
1571 F	N′-phenyl-N′-ethyl-N-diethylethylenediamine	
Thymoxyethyldiethylamine Tastromin 929 F	2-Isopropyl-5-methyl phenoxyethyldiethyl-amine	
Benadryl Diphenhydramin A424 S-51	β-dimethylaminoethyl benzhydryl ether	
Linadryl A 446	β-morpholinoethyl benzhydryl ether	
Pyribenzamine Tripelennamine 63C	N′-pyridyl-N′-benzyl-N-dimethylethylenediamine	

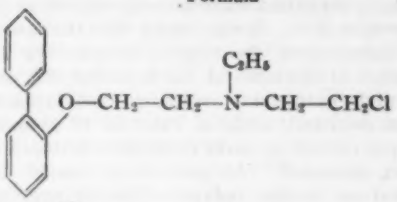
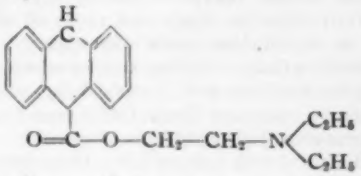
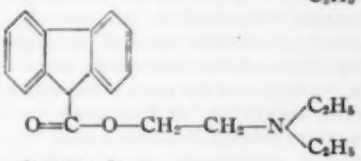
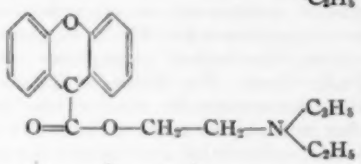
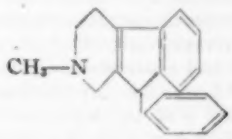
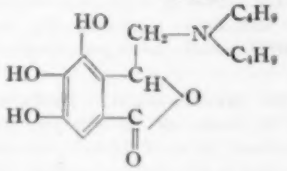
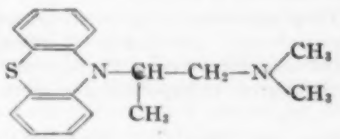
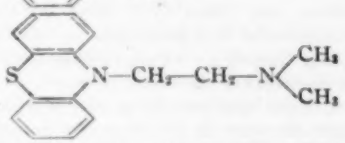
(*Cont'd on p. 386*)

TABLE III.—CHEMICAL STRUCTURE OF SOME ANTIHISTAMINES (*Cont'd from p. 385*)

Common Name	Chemical Name	Formula
2325 RP	N′-phenyl-N′-ethyl-N-dimethylethylenediamine	
Antergan 2339 RP 97 B	N′-phenyl-N′-benzyl-N-dimethylethylenediamine	
Antistine	N′-phenyl-N′-benzyl aminomethylimidazoline	
Heteramine	N′-benzyl-N′-2-pyrimidyl-N-dimethylethylenediamine	
Neoheteramine	N′-*p*-methoxylbenzyl-N′-2-pyrimidyl-N-dimethylethylenediamine	
Neoantergan Pyranisamine Anthisan 2786 RP	N′-*p*-methoxybenzyl-N′-pyridyl-N-dimethylethylenediamine	
Thenylene Histadyl Methapyrilene AH-42 Lilly-01013	N′-pyridyl-N′-thenyl-N-dimethylethylenediamine	
Bromothen	N′-pyridyl-N′-5-bromothenyl-N-dimethylethylenediamine	
Chlorothen	N′-pyridyl-N′-5-chlorothenyl-N-dimethylethylenediamine	
	α-Naphthylmethylethyl-β-chloroethylamine	

(*Cont'd on p. 387*)

TABLE III.—CHEMICAL STRUCTURE OF SOME ANTIHISTAMINES (*Cont'd from p. 386*)

Common Name	Chemical Name	Formula
	β-2-biphenoxyethyl-β-chloroethylethylamine	
D	Diethylaminoethyl-9,10-dihydroanthracene carboxylate	
Pavatrine F	Diethylaminoethyl-7-fluorene carboxylate	
X	Diethylaminoethyl-9-xanthene carboxylate	
Thephorin Nu 1504	2-Methyl-9-phenyl-2,3,-4,9-tetrahydro-1-pyridindene	
Anthallan	3′-Di(*n*-butyl)aminomethyl-4,5,6-trihydroxybenzo-(1,2c)furan-1′-(3′)-one	
3277RP	N-dimethylamino-isopropylthiodiphenylamine	
3015 RP	N-dimethylaminoethylthiodiphenylamine	

GENERAL PHARMACOLOGY

Effect on Blood Pressure and Respiration.—Winder and Thomas (57) have shown that Benadryl intravenously given in phenobarbitalized dogs at a rate of 1–43 mg./Kg. per minute (1–16 mg./Kg.) produced a primary vascular depression of about two minutes' duration. This blood pressure fall varies from 0.05% to 19% of the previous level. A secondary rise in blood pressure of about forty-six minutes' duration occurred after the primary fall but was often masked by the primary depression. Both phases were found to be independent of vagal cardiac innervation, important arterial reflex zones, heart rate changes, peripheral cholinergic mechanisms, and nervous or mechanical respiratory changes. Variable slight cardiac acceleration, independent of the blood pressure changes, was observed with the low doses, while larger doses showed a primary deceleration. Electrocardiographic records showed no gross disturbances in cardiac rhythm. In doses of 50 μg., Benadryl caused a slight to moderate decrease in the

amplitude of systolic contractions of the perfused rabbit heart; at the same time a transient increase followed by a persistent mild decrease was observed in the coronary flow. It was shown that the rate of injection determined the type of respiratory response seen. At the rates of 1.2 to 43 mg./Kg. per minute a slight to moderate, transient stimulation of respiration occurred, while at rates of 19 or more mg./Kg. per minute an early restriction in depth of respiration occurred. Larger doses caused an acceleration or intense polypnea lasting over an hour. The restriction in depth and most of the acceleration in respiration were eliminated by vagotomy and when the carotid innervation was also removed, respiration was not disturbed by large doses. Previously Page and Green (58) found that dogs under pentobarbital anesthesia responded to intravenous Benadryl with a sharp fall in blood pressure and a cessation of respiration. However, pulse pressure remained full despite the low blood pressure. They also observed that there was a reduction in the pressor responses of tyramine, epinephrine, barium chloride, angiotonin, and histamine after Benadryl. In the perfused isolated rabbit ear a greatly diminished vasoconstrictor response was seen when these drugs were injected with Benadryl. In both of these experiments epinephrine was difficult to block. Loew, MacMillan, and Kaiser (59) have shown that epinephrine is potentiated by Benadryl. After intravenous doses of Benadryl (3 mg./Kg.), 10 μg. of epinephrine gave an average response of 64 mm. Hg, whereas the same dose without premedication gave an average response of 45 mm. Hg. However, Benadryl had a depressor action in larger doses, and doses of 1 mg./Kg. gave decreases of 5–10 mm. Hg and doses of 3 mg./Kg. gave decreases of 10–30 mm. Hg. Dose of 1 mg./Kg. had no effect on respiration and doses of 3 mg./Kg. increased the rate 20–40% usually for a period of one to three minutes.

Yonkman, *et al.* (60) have shown that Pyribenzamine intravenously in doses of 10–200 μg./Kg. caused minor fluctuations in cats' blood pressure after the small doses and a sustained rise after the larger doses. They also found that Pyribenzamine potentiated epinephrine. Previously Yonkman, *et al.* (61), found that intravenous Pyribenzamine produced variable degrees of hypotension in normal dogs.

Lee, Dinwiddie, and Chen (45) observed that Thenylene administered intravenously in cats at doses of 1 mg./Kg. caused a very slight hypotension followed by an elevation in the blood pressure. The drug also potentiated the pressor action of epinephrine. A slight decrease in the depth of respiration was also seen. This blood pressure effect has been confirmed (62).

Loew, Micetich, and Achenbach (63) showed that benzhydryl-ethyl-β-chloro-ethylamine intravenously in cats and dogs in doses of 10 mg./Kg. reversed the pressor response of epinephrine. Further, a 10 mg./Kg. dose of this drug to unanesthetized dogs prevented the hypotension and/or the pressor response to epinephrine for seventy-two hours. Similar results were reported for another drug of this series, alpha-naphthylmethylethyl beta-chloroethylamine (64). These epinephrine pressor reversal and blocking effects are similar to those originally reported for the first antihistamine compounds.

Halpern (42) reported that 2325 RP in doses of 10 mg./Kg. caused a rise in blood pressure of 20–60 mm. with a duration of five to twenty minutes. Antergan in a dose of 5 mg./Kg. under the same conditions had no effect. In chloralose dogs, both compounds produced apnea, then respiratory acceleration. Acceleration and a more profound respiration was observed in unanesthetized dogs. Neither compound modified the effect of epinephrine on cardiac tissue. Parrot (65, 66) has confirmed the hypertensive action of Antergan in both dogs and cats.

Bovet, Horclois, and Fournel (67) found that intravenous doses of Neoantergan 1–5 mg./Kg. produced a slight rise in the dogs' blood pressure, larger doses caused hypotension and doses of 50 mg./Kg. death by cardiovascular collapse.

Lehmann and Knoefel (68) have investigated diethylaminoethyl-fluorene-9-carboxylate and found that doses of 0.05 mg./Kg. intravenously in dogs had no effect upon either blood pressure or respiration. In the spinal dog doses of 2 mg./Kg. gave the same results. It was 130 times less effective than atropine in counteracting the depressant effect of acetylcholine in the dog. In further work with this compound and with diethylaminoethyl-xanthene carboxylate and diethylaminoethyl-9,10-dihydroanthracene-9-carboxylate (69) they confirmed their previous work on the first compound, and showed that the dihydroanthracene compound had a hypotensive effect at a dose of 0.5 mg./Kg. This compound also reduced and at times reversed the pressor effect of epinephrine in etherized cats.

Minard and Rosenthal (80) reported that subcutaneous injection of 40 mg./Kg. of thymoxyethyldiethylamine caused an increase in pulse and blood pressure as well as a transitory rise in hemoglobin level. The peak occurred about thirty to forty-five minutes after injection and there was a gradual return to normal.

Anthallan has only a slight vaso-pressor effect in animals in small doses but doses approaching the LD_{50} cause a profound blood pressure fall. Respiration is stimulated only with large doses (81).

Dreyer and Harwood (82) reported that Neoheteramine (1–5 mg./Kg.) caused an immediate fall in blood pressure without a change in heart rate in atropinized cats and dogs under urethane or urethane and chloralose anesthesia. In the Straub heart Neoheteramine caused diminished systolic contractions. Cardiometric measurements in the dogs confirmed this action of the drug. Slight transient dilatation of the perfused rabbit's ear was also seen after Neoheteramine. No potentiation of epinephrine response could be demonstrated on either the blood pressure of the cat or on the isolated uterus of the rabbit.

Reuse (130) observed an increased coronary flow and a depression of the force and rate of beat in the perfused cat heart due to Antergan, Neoantergan, 3277 RP, and Benadryl. Similar results were obtained with the isolated rabbit heart.

Autonomic Effects.—Dreyer (83) reported that, in cats, 1.5 mg./Kg. of Benadryl intravenously caused a diminution of salivary flow on chorda tympani stimulation but not on cervical sympathetic ganglion stimulation. The former was completely blocked with a dose of 5 mg./Kg. but the latter only partially blocked. Motor response to vagal stimulation also was partially blocked.

Yonkman, *et al.* (60, 61), found that Pyribenzamine blocked the salivary secretogogue effect of histamine but had no effect on salivation produced by pilocarpine, epinephrine, or stimulation of the chorda tympani or the cervical sympathetic ganglion. Further, Pyribenzamine potentiated the effect of epinephrine on the nictitating membrane of the cat.

Halpern (42) reported that 2325 RP and Antergan both increased salivary secretion and decreased urinary secretion in dogs. Also neither compound modified the effect of epinephrine or acetylcholine on cardiac tissue or on the autonomic nervous system.

Chauchard and Chauchard (86) showed that Antergan, 2.5% applied locally, decreased the chronaxia of the cervical sympathetic system and at times blocked conduction. Upon the post-ganglionic fibers of that system the reverse was true.

Neoheteramine has no effect on the blood pressure response elicited by sympathetic stimulation in the cat. Some blocking of the chorda tympani response in cats was seen but with larger doses (8 mg./Kg.) did not abolish the secretion. Neoheteramine partially prevented electrically induced vagal stimulation in the cat but had no effect upon the vagal effects on the intestine. At times intestinal vagal contractions were potentiated by the drug (82).

Effect on the Intestinal and Genitourinary Systems.—Loew, *et al.* (59), reported that while Benadryl and the quaternary ammonium derivative of Benadryl were effective in antagonizing the spasmogenic action of acetylcholine on the isolated guinea-pig ileum they were far less effective than atropine in this action. Against barium-induced spasm both compounds were less effective than Pavatrine or Trasentin. 1571 F under the same conditions was comparatively impotent. Sherrod, Schloemer, and Loew (70, 71) found that when equal doses (3 mg./Kg.) of Benadryl, Pyribenzamine, or Neoantergan were administered intravenously to dogs there was a reduction in tone and an inhibition of the spontaneous activity of the duodenum with the former and an increase in duodenal tone with the latter two compounds. The duration of action was about thirty minutes regardless of the drug given. As acetylcholine had little or no effect on the duodenum *in situ* there was no possibility of demonstration of an *in vivo* anticholinergic effect by the three drugs. None of the drugs had any effect on the uterus *in vivo*. Schild (136) found Benadryl more potent than Neoantergan in counteracting acetylcholine spasm of the isolated guinea pig ileum. However, if the drugs were left in contact with the tissue the latter compound showed a greater potency.

Hoekstra and Steggerda (72), using thorium dioxide as an opaque medium and a tambour system, found that intracardial Pyribenzamine (0.1–2.9 mg./Kg.) increased the amplitude and frequency of colonic activity in proportion to the amount administered. In these experiments with six dogs an increase in intestinal muscular tone was also observed. Kaiser and Reynolds (73) reported that there was an increase in the frequency and duration of the contractions of the endometrial blood vessels of intraocular transplants in the anterior chamber of the rabbit after Pyribenzamine. The drug was administered by instillation into the conjunctival sac. The effect observed could have been due to a milking action rather than to any estrogenic effect of Pyribenzamine.

Thenylene has a very weak antispasmodic action on the rabbit intestine when compared with papaverine against barium chloride and atropine against acetylcholine (62). Lee, *et al.* (45), observed that 1:50,000 to 1:20,000 concentrations of Thenylene caused contraction of the virgin guinea-pig uterus. Further, they found that on the guinea-pig intestine the drug antagonized Mecholyl but had only about $^1/_{200}$ the effect of atropine.

Halpern (42) reported that 100 gamma of 2325 RP slightly relaxed the isolated rabbit intestine without affecting its rate of contraction while the same dose of Antergan was similar to epinephrine in its effect. Both compounds caused contraction of the isolated guinea-pig uterus. Mayer (79) found Antergan and Pyribenzamine about equal in counteracting the acetylcholine induced spasm of the guinea-pig ileum *in vitro*.

Lehmann and Knoefel (68) found that diethylaminoethyl-fluorene-9-carboxylate in doses of 1.12×10^{-7} to 8×10^{-8} counteracted acetylcholine 10^{-6} when tested on the isolated ileum. The drug also counteracted barium 10^{-4} in doses of 8×10^{-6} to 4×10^{-6}, and had a relaxant effect upon the dog and rabbit intestine *in vivo* at doses of 0.1 mg./Kg. and 0.05 mg./Kg. The normal dog uterus and the detrusor of the urinary bladder were relaxed *in situ* with doses of 2 mg./Kg. The guinea-pig ileum *in situ* was also relaxed. Further work on this drug and two others, diethylamino-xanthene carboxylate and diethylaminoethyl - 9,10 - dihydroanthracene - 9 - carboxylate, showed that the anthracene compound was more effective than either of the others against acetylcholine spasm. The xanthene derivative was less effective than the others against barium spasm of the rabbit ileum. The anthracene compound alters the effect of epinephrine on the isolated rabbit and human uterus. Both the xanthene and the anthracene compounds relaxed the rabbit ileum *in situ* (69). Lehmann (74) studied the anthracene and fluorene compounds further and found that the rabbit uterus *in situ* and *in vitro* was relaxed by doses of 3–5 mg./Kg. of the former while the latter caused contraction. Upon the isolated human uterus both compounds caused relaxation of both the circular and longitudinal muscles.

Rosenthal and Brown (78) found that thymoxyethyldiethylamine 2 mg. reduced but did not abolish the intestinal spasm of guinea-pig ileum caused by acetylcholine 0.01 mg.

Anthallan stimulates the isolated intestine and uterus in high doses. No gastrointestinal symptoms (nausea, vomiting, diarrhea, stool consistency or frequency) were observed even in lethal doses (81).

Reuse (130) showed that Neoantergan and Antistine were about equal in counteracting acetylcholine spasm of the guinea-pig ileum. Further, both drugs were more potent than 3277 RP. Neoantergan and 3277 RP had no effect on the isolated nonpregnant cat uterus and both drugs counteracted acetylcholine but at higher doses than were required to counteract histamine. Similar results were obtained on the pregnant and nonpregnant rabbit uterus and virgin guinea-pig uterus with Neoantergan, 3277 RP, Antistine, and Benadryl.

Lehmann, *et al.* (75), reported that Thephorin counteracted the spasmogenic effect of acetylcholine and barium on the isolated guinea-pig intestine.

Shock.—Ingraham and Wiggers (85) showed that Benadryl 15 mg./Kg. subcutaneously thirty minutes before hemorrhage and 5 mg./Kg. as a second dose one hour later had no effect on hemorrhagic shock in dogs. All the criteria of irreversible shock were seen and the authors concluded that histamine plays no part in the irreversible tissue damage of hemorrhagic shock.

Jourdan and Chatonnet (84) found that 10 mg./Kg. of Antergan had no effect on the development of experimental traumatic shock and sometimes made it worse.

Anesthetic and Analgetic Effects.—Reuse (130) has shown that 3277 RP, Benadryl, Neoantergan, and Antistine produced local anesthesia in the frog lumbar plexus preparation and that the first two compounds were the most potent.

Halpern (42) found that 2325 RP had a local anesthetic action in animals.

Dews and Graham (97) reported that Neoantergan had a slight analgetic effect in rats in doses of 12 mg./Kg. and that when the dose was increased to 100 mg./Kg. a greater analgesia as well as a narcotic effect was seen. Graham (94) showed by the guinea-pig wheal test that Neoantergan was 3.3 times as strong, Benadryl 2.5 times as strong, and Antistine 1.5 times as strong as procaine.

Lehmann and Knoefel (68) found that diethylaminoethyl-9-fluorene carboxylate and diethylaminoethyl - 9,10 - dihydroanthracene-carboxylate were equal in anesthetic value when tested on the rabbit cornea. Diethylaminoethyl-xanthene-9-carboxylate also has an equivalent local anesthetic effect (69).

Burchell and Varco (127) reported that large subcutaneous doses of thymoxyethyldiethylamine lowered sensitivity to pain but did not produce complete analgesia in dogs.

Effect on the Excised Trachea.—Castillo and de Beer (76, 77) have shown that Benadryl, $1:15 \times 10^6$, counteracted acetylcholine induced contraction of the normal excised guinea-pig tracheal chain. However, Benadryl had no effect on the normal tracheal chain nor did it have any effect upon barium-induced contraction.

Acute Toxicity.—In general, all of the antihistamines present the same train of symptoms when administered in toxic doses to various animals. Benadryl and its β-morpholinoethyl derivative produced violent excitement, convulsive attacks, prostration, and death by respiratory and cardiac failure (50, 88). In dogs an intravenous dose of 5 mg./Kg. caused a slight spasticity, apprehension, and occasionally a clonic convulsion of short duration. The morpholine derivative responded similarly at a 15 mg./Kg. dose but not at lower doses. Violent excitement, prostration, and convulsions were caused by both compounds at a dose of 20 mg./Kg. Other symptoms seen were dilated pupils, exaggerated somatic reflexes, increased sensitivity to sound, and painful sensations of the skin and feet. The reactions of all animals were the same regardless of the route of administration but the onset varied in rapidity. However, in dogs Benadryl had a longer duration of action than the morpholine compound. Although tolerance did not develop in dogs with daily doses of 10 mg./Kg. of Benadryl for twelve days the duration of the symptoms decreased. Sedation with barbiturates thirty to ninety minutes before intravenous injection of either compound prevented excitement and convulsions but did not lessen respiratory-cardiac depression or decrease the mortality (87).

Pyribenzamine presents the same preliminary symptoms in most animal species but at times a secondary depression intervened between excitement and death. In dogs surviving intravenous doses of 3 mg./Kg. the marked excitement and salivation gradually decreased until recovery. Local necrosis of the skin was seen in the rats surviving subcutaneous doses and fibroblasts replaced the muscle cells of the gastroenemii of rats injected intramuscularly (90). The castration of female rats brought their LD_{50} values up to those of the males. Estrogen therapy did not appear to cause a return to greater vulnerability (90, 91).

Neter (93) showed that Benadryl, Pyribenzamine, and Heteramine were toxic to embryonic chick hearts. Benadryl and Pyribenzamine in amounts of 2.5 mg. caused immediate bradycardia and stoppage within two minutes. Hemorrhage was sometimes seen at this concentration but when the concentration was decreased (0.1 to 0.0001 mg.) there was no effect. Heteramine had a similar action depending upon the concentration used.

Halpern (42) reported that toxic doses of both 2325 RP and Antergan caused psychomotor excitement, tetanic convulsions, polypnea, locomotor ataxia, opisthotonis, and death by asphyxia. In dogs the psychomotor effects last from 0.5 to 2 hours.

Graham (94) compared the toxicity of three antihistamines and found that Neoantergan acted at once to cause generalized tremor, increased activity, incoordination of movement, Straub tail phenomenon, squeaking, restlessness, clonic convulsions, and death by asphyxia. Benadryl was similar but less violent; no tail phenomenon was observed but incoordination and excitement were of longer duration and death occurred from general exhaustion. Antistine caused slight motor incoordination, weakness, and marked depression which usually ended with asphyxial convulsions due to depression of vital centers and respiratory paralysis.

Litchfield, *et al.* (54), compared the acute toxicity of Chlorothen, Bromothen, Thenylene, and Pyribenzamine and reported the following symptoms: excitement, tremors, convulsions, tonic and clonic muscle spasms, and respiratory irregularities. At a dose of 2 mg./Kg. intravenously every twenty minutes, a dog showed some of the above toxic symptoms and died after receiving a total of 18 mg./Kg. of Pyribenzamine. Bromothen at twice the dose produced a similar effect but the death did not occur even after a total dose of 36 mg./Kg. See Table IV for the LD_{50} of the various compounds.

Chronic Toxicity.—Gruzhit and Fishen (50) studied the chronic toxicity of Benadryl and its morpholine derivative. They found that mice gained weight at the same rate as the controls when they were fed the morpholine compound as 0.1% of their diet, whereas the Benadryl-fed mice did not. Greater percentages of both compounds caused weight losses in all the mice. Rats gained weight when Benadryl up to 0.25% of the diet was fed but above that level there was a weight loss. Dogs given both compounds in single daily doses of 10 mg./Kg. showed a slight fluctuation in body weight, but there was no disturbance in their hematology or total blood NPN; also the urine was free of sugar

TABLE IV.—LETHAL DOSES OF VARIOUS ANTIHISTAMINIC DRUGS

Drug	Animal	Route Given	LD_{50} ± S. E., Mg./Kg.	Reference
Benadryl	Mouse	Oral	164	50
			270	51
			167	89
	Mouse	Subcutaneous	127	50
			130	89
	Mouse	IP	98	50
			82	89
			86	51
			140 ± 13	160
	Mouse	IV	27.5	51
			18.26 ± 0.6	62
	Rat	Oral	500	50
			545	89
	Rat	Subcutaneous	474	50
	Rat	IP	61 ± 9.0	88
	Rat	IV	42	50
			45.7	89
	Rabbit	IV	10	50
			10.5	89
			17	89
	Guinea Pig	IP	125	51
	Dog	IV	24	50
			30	50
β-morpholinoethyl benzhydryl ether A446	Mouse	Oral	327	50
		Subcutaneous	440	50
		IP	185	50
	Rat	Oral	916	50
		IV	35	50
	Rabbit	IV	21	50
	Dog	IV	70	50
Pyribenzamine	Mouse	Oral	210	94, 51
			200 ± 14.0	141
	Mouse	Subcutaneous	75	92
	Mouse	IV	12	92
			9	51
			29.7 ± 1.67	62
	Mouse	IP	82	51
			62 ± 7.0	141
			68 ± 7.0	160
	Rat M	Oral	570	92
	F	...	515	92
	Rat M	Subcutaneous	340, 350	92
	F	...	225, 250	93
	Rat M	IV	12	92
	F	...	12	92
	Rabbit	Subcutaneous	33	92
		IV	27 ± 3.0	141
		IV	9	92
	Dog	IV	3–4	92
		Oral	20–30	141
Thenylene	Mouse	Oral	182.2 ± 12.8	62
			190	63
	Mouse	Subcutaneous	60	63
	Mouse	IP	90	63
			79 ± 12	141
	Mouse	IV	13	63
			19.85 ± 0.69	62
	Rat	Oral	450	63
		IP	70	63
	Guinea Pig	Oral	250	63
		Subcutaneous	25	63
			70	63
	Cat	Oral	150	63
		IP	55	63
	Dog	Subcutaneous	25	63
		IV	10	63
2325 RP	Rat	Oral	115	66
		IV	50	66
	Dog	IM	20–25	66
		IV	10	66

(*Cont'd on p. 392*)

TABLE IV.—LETHAL DOSES OF VARIOUS ANTIHISTAMINIC DRUGS (*Cont'd from p. 391*)

Drug	Animal	Route Given	LD_{50} ± S. E., Mg./Kg.	Reference
Antergan	Rat	Oral	30	66
		IV	17.5	66
	Dog	IM	10	66
		IV	5	66
Neoantergan	Mouse	IV	30	94
		IP	102 ± 11	160
Diethylaminoethyl-9,10-dihydroanthracene carboxylate	Mouse	IP	150	70, 71
Diethylaminoethyl-9, xanthene carboxylate	Mouse	IP	250	71
Diethylaminoethyl-9, fluorene carboxylate	Mouse	IP	320	70
	Rat	IP	211 ± 19	88
Anthallan	Mouse	Oral	3200–2700	82
		Subcutaneous	1300	82
		Rectal	650	82
		IP	285	82
		IV	77	82
	Guinea Pig	Oral	2700–2750	82
		Subcutaneous	600	82
	Rabbit	Oral	2000	82
	Cat	Oral	2000	82
		IV	50	82
	Dog	Oral	2325	82
		IV	83	82
	Monkey	Oral	100	82
Heteramine	Mouse	Oral	300–400	91
		Subcutaneous	62.5–75	91
		IP	76 ± 7	160
Thephorin	Mouse	Oral	280	141
		Subcutaneous	300	141
				141
		IP	88	141
		IV	225	141
	Rat	Oral	280	51
	Guinea Pig	IP	125	51
	Rabbit	Oral	500	51
		IV	17	51
	Dog	...	33	51
Antistine	Mouse	Oral	150	51
Chlorothen	Mouse	Oral	390 ± 27	141
		IP	112 ± 10	141
	Rabbit	IV	77 ± 14	141
Bromothen	Mouse	Oral	430 ± 25	141
		IP	130 ± 6	141
	Rabbit	IV	79 ± 7	141
	Dog	Oral	90–120	141
3015 RP	Mouse	IP	140 ± 13	160
3277 RP	Mouse	IP	190 ± 21	160

and albumin. Some acute toxic phenomena (emesis, irritability, etc.) were seen with doses of 25 and 40 mg./Kg. of Benadryl while the morpholine compound caused a slight lowering in the red cell count and hemoglobin values at 40 mg./Kg. A dose of 60 mg./Kg. of the latter gave results similar to the 40 mg./Kg. dose of the former. Weight loss, persistent anorexia, decreased red cell counts and hemoglobin values, and mild albuminuria were seen when 80 mg./Kg. of the morpholine compound was given. However, total blood NPN remained normal. Daily subcutaneous injections for twenty-one days of 2.5, 5.0, and 7.5 mg./Kg. of Benadryl caused a weight loss in guinea pigs. Both compounds upon prolonged administration to small animals produced cachexia, gastrointestinal atony, congestive stasis of visceral organs, and superimposed inflammatory foci in the lungs. The thyroid glands showed mild depletion of colloid substance and a mild hypertrophy of the acinal cells. Large animals showed no parenchymatous tissue changes. Chen and Abreu (89) found that CCl_4 poisoned rats detoxified Benadryl (141 mg./Kg.) in the same manner as the normals.

Rieveschl and Gruhzit (88) reported that Benadryl administered orally in doses of 10, 25, 40, and 60 mg./Kg. per day to mice caused no changes in blood counts, hemoglobin values, NPN values, no change in eating habits or any significant weight change. Doses of 10–25 mg./Kg. in dogs produced no acute or chronic degenerative changes in the liver, spleen, kidney, pancreas, adrenals, heart, brain, gall bladder, urinary bladder, or gastrointestinal mucosa.

Further, there was no congestion, edema, petechial hemorrhages, or necrotic cells in the central nervous system tissues.

Koepf, Arbesman, and Munafo (95) reported that Pyribenzamine administered orally to dogs in doses of 50 to 100 mg. daily for a period of one year had no effect upon body weight, kidney function, liver function, blood urea nitrogen, blood creatinine values, hematocrit, hemoglobin values, red cell, white cell, and polymorphonuclear leucocytes. Mayer, *et al.* (90), working with rats of both sexes reported the same results after the oral administration of 5 mg./Kg. per day for five months. Further, they found no changes in appetite, reproductive capacity, gastrointestinal function, general appearance, or comport. Mathieson, *et al.* (91), reported the same results and further stated after five weeks of similar treatment the offspring of such animals were entirely normal. Hays, *et al.* (96), fed Pyribenzamine, 3 mg./Kg. per day, for six weeks to male and female rats. With a normal caloric intake there was no difference between medicated and nonmedicated animals. With a reduced caloric intake, the controls and medicated animals showed a 50% loss in body weight as compared to the normally fed controls. However, this loss was due to reduced caloric intake and not to the Pyribenzamine. There were no changes in red cell, white cell, or hematocrit values.

Lee, *et al.* (45), found no pathological lesions, grossly or microscopically, after feeding 10 mice 20 mg./Kg. of Thenylene daily for four weeks. Hazel (62) reported that rats receiving Thenylene as 0.05 to 1% of their diets for more than five months showed normal growth and activity.

Dews and Graham (97) gave Neoantergan in doses of 35 mg./Kg. subcutaneously twice daily and observed arrested growth on the seventh day. When the drug was discontinued normal growth resulted. The administration of 16 mg./Kg. daily had no effect upon growth. Graham (94) found that the daily subcutaneous administration of 20 mg./Kg. of Neoantergan, Antistine, or Benadryl had no effect upon the weight gain of weanling mice.

Pogge (98) stated that rats receiving 10 mg./Kg. of Neoantergan daily for six months and up to 200 mg./Kg. daily for thirty-two days showed no signs of chronic toxicity nor was there any gross or microscopic evidence of abnormalities due to the drug. Similar results were seen in dogs receiving 20 mg./Kg. of the drug five times weekly for six months and in monkeys receiving 50 mg./Kg. daily for thirty-five days.

Strauss (51) could find no evidence of toxicity due to Thephorin when fed in a daily dose of 35 mg./Kg. to rats for fifteen weeks. Growth, red cell, white cell, and hemoglobin values were unchanged and the histological examination of the various organs revealed no pathological lesions.

Litchfield, *et al.* (54), fed two male and two female dogs 30 mg./Kg. of Bromothen daily for sixty days. There was no loss in body weight, change in feeding habits, or changes in hematological characteristics. However, salivation occurred in all animals and this became a conditioned reflex in the two males after twenty-five days. No changes were observed in two groups of mice at 20 and 160 mg./Kg. Bromothen fed for thirty days. Similar results were obtained with weanling rats at a dosage level of 65 mg./Kg. but at 700 mg./Kg. the animals had a depressed daily food intake and a 30% less weight gain than the controls. No gross pathology was found in any of the animals.

Anthallan administered to mice, rats, and guinea pigs in doses from 15 to 500 mg./Kg. for periods up to fourteen months had little or no effect upon body weight gain or upon the breeding characteristics or fertility of the animals. No gross or microscopic pathological lesions were found (81).

Feinstone, *et al.* (46), observed no effect upon the weight or hemoglobin values of weanling rats fed 50 mg./Kg. or subcutaneously injected with 20 mg./Kg. of Heteramine daily for thirty-five days.

HISTAMINE BLOCKING ACTION

Effect on Blood Pressure.—Wells, *et al.* (101), reported that the depressor effect of 0.001–0.002 mg./Kg. of histamine on the blood pressure of dogs was abolished by 3 mg./Kg. of Benadryl. After 0.016–0.032 mg./Kg. of histamine this blocking effect was not as marked. They found that a direct proportionality existed between the dose of histamine injected and the amount of this dose antagonized by a given amount of Benadryl. The maximum inhibition (94%) was obtained with 4 mg./Kg. of Benadryl.

In dogs, 1–3 mg./Kg. of Benadryl significantly reduced the depressor response elicited by 4–15 μg. of histamine. These reductions of 58% to 68% showed that Benadryl had an optimum effect at the lower dose (59).

Benadryl has been shown to be equal to Pyribenzamine and Neoantergan in counteracting the depressor effect of 5–10 μg./Kg. of histamine diphosphate (71).

Page and Green (58) reported that Benadryl easily blocked the depressor response of the dog to intravenous histamine. Further, a similar blocking action was observed in the perfused rabbit's ear. However Benadryl did not completely block the response to injected dog or rabbit serum.

Wells, Morris, and Dragstedt (152) found that 10 mg./Kg. of Benadryl significantly reduced the vasodepressor effects of a shocking dose of horse serum in sensitized dogs. It was shown, by the cat blood pressure assay, that 10 μg. of histamine were liberated in one of the test animals but it could not be concluded that histamine was the only vasodepressor agent liberated in shock.

Yonkman, *et al.* (102), found that 3 mg. of Pyribenzamine intravenously in dogs counteracted the hypotensive effect of 15 μg. of histamine, but had no effect upon the hypotension caused by 1 cc. of horse serum intravenously in sensitized animals.

Yonkman, *et al.* (252), showed that Pyribenzamine in doses of 1–3.5 mg./Kg. in sensitized dogs prevented anaphylactic shock due to the injection of antigen. Very slight decreases in blood pressure occurred but the pulse pressure remained relatively constant. Respiration was increased.

Thenylene, 1 mg./Kg. intravenously in cats, abolished the depressor effect of 0.4 μg. of histamine by the same route and significantly reduced the response to larger doses of histamine. An oral dose of 15 mg./Kg. of Thenylene completely abolished the response to 0.5 μg. of injected histamine. Premedication with 5 μg./Kg. of Thenylene intravenously re-

duced the depressor response caused by 0.1–0.3 μg. of histamine by at least 50% (62).

The depressor effect of histamine on the blood pressure of dogs is diminished or annulled by alpha-naphthylmethylethyl-β-chloroethylamine HCl (64).

The vasodilatation, hypotension, and shock caused by 0.25–3 mg./Kg. of intravenous histamine in chloralose or urethane dogs was counteracted by 10 mg./Kg. of 2325 RP or 5 mg./Kg. of Antergan (42).

In the rabbit, 5 mg. of Neoantergan partially reduced the depressor response caused by 0.25 mg. of histamine and abolished entirely the respiratory stimulation usually seen after histamine. In chloralose cats, Neoantergan had no effect on the blood pressure fall due to histamine, but did abolish the action of histamine on the spleen. However, in etherized cats Neoantergan significantly reduced the depressor effect of histamine. Neoantergan also reduced the vasodilator effect of histamine in the dog hind limb preparation. Histamine constriction of the blood vessels of the perfused rabbit's ear was significantly reduced by premedication with 0.001 μg. of Neoantergan and abolished with 0.01 μg. of the drug (97).

Bovet, Horclois, and Fournel (67) observed that premedication with 0.1 mg./Kg. of Neoantergan diminished or abolished the depressor effect of small and moderate doses of histamine in dogs.

When compared by the cat blood pressure method the depressor effect of 2.5 mg./Kg. of histamine was reduced by 50% by 0.14 mg./Kg. Neoantergan, 1.3 mg./Kg. Antistine, or 1.7 mg./Kg. Benadryl (108).

Lehmann and Knoefel (69) showed that diethylaminoethyl-9,10-dihydroanthracene carboxylate, 0.5 μg./Kg., decreased by 50% the depressor effect of 1 and 2 μg./Kg. of histamine on the cat's blood pressure.

Ramanamanjary (113) found that subcutaneous premedication with 5 mg./Kg. of 929 F, 20 mg./Kg. of 1571 F, 1 mg./Kg. Antergan, or 0.5 mg./Kg. Neoantergan prevented the depressor effect of 0.2 mg./Kg. of histamine on the rabbit blood pressure. Antergan and Neoantergan were not effective when chloralose, barbiturates, or urethane was the anesthetic.

Minard and Rosenthal (80) reported that thymoxyethyldiethylamine, 40 mg./Kg. subcutaneously reduced the degree and duration of hemoconcentration but not the vasodepressor effect of 1 mg./Kg. of histamine.

Marsh and Davis (49) confirmed previous reports that thymoxyethyldiethylamine, 1571 F, Benadryl, and Antergan counteracted the vasodepressor effects of intravenous histamine in dogs. In equal doses 4.4 mg./Kg. the drugs rated as follows: Benadryl 125, Antergan 80, Pyribenzamine 40, thymoxyethyldiethylamine 12, and 1571 F 8. Similar ratings were obtained at higher doses of Benadryl and Antergan. All the compounds antagonized the effect of histamine on the rabbit blood pressure response and no differences in potency could be determined. The drugs also antagonized the vasodepressor effects of histamine in rats and guinea pigs.

Dreyer and Harwood (82) reported that Neoheteramine (2–5 mg./Kg.) counteracted the vasodepressor effect of 1–2 μg. of histamine, but larger doses of histamine, 5–7 μg./Kg., could not be offset by 8–10 mg./Kg. of the former. However, the vasoconstrictor effect of histamine on the perfused rabbit's ear was counteracted by Neoheteramine.

Lehmann, *et al.* (75), reported that Thephorin either abolished or prevented the vasodilator effect of histamine in normal cats. In decapitated cats the vasopressor response to histamine was blocked. Thephorin reversed the blood pressure effect seen when histamine was injected into rabbits.

Halpern (131) found that 3277 RP completely blocked the vascular effects of 0.1–1 mg./Kg. of histamine in the urethanized rabbit and the hypotensive effects in the dog and cat.

Experimental Anaphylaxis.—Loew and Kaiser (122) passively sensitized guinea pigs with rabbit antiovalbumin serum, then injected intrajugularly 2 cc. of a 2% solution of crystalline ovalbumin twenty-four hours later. Three milligrams of Benadryl protected 92.9%, 3 mg. of 1571 F protected 85%, and 12 mg. of β-morpholinoethyl benzhydryl ether protected 100% of the animals. Selle (123) confirmed the protective action of Benadryl by his work with egg albumin shock.

Mayer, *et al.* (149), found that although 2% aerosol of Pyribenzamine protected sensitized guinea pigs against the immediate toxic effects of a shocking dose of antigen, many of the animals died within twenty-four hours. This toxic effect was shown to be due to the antigen because there was no evidence of lung damage in animals treated with the drug alone.

Mayer and Brousseau (151) reported that Pyribenzamine in doses of 10–50 mg./Kg. protected sensitized mice from the lethal effects of a shocking dose of antigen.

Arbesman, *et al.* (227), found that 1, 1.5, and 3 mg./Kg. of Pyribenzamine intraperitoneally in sensitized guinea pigs protected the majority of the animals against anaphylactic shock produced by the intracardial injection of 1 cc. of human serum. The protective effect was greater with larger doses.

Marcus (135) reported that premedication with 5–10 mg. of Pyribenzamine or Benadryl ten to thirty minutes before a shocking dose of antigen protected guinea pigs against 8 lethal doses of antigen. Similar results were obtained by Mayer (142) with lower doses (2.5–5 mg./Kg.) of Pyribenzamine.

Achenbach and Loew (64) observed that the subcutaneous injection of 0.025 to 0.1 mg./Kg. of alpha - naphthylmethylethyl - β - chloroethylamine HCl prevented anaphylactic shock in guinea pigs.

Vallery-Radot, *et al.* (117), showed that Antergan at a dose of 10–20 mg./Kg. prevented serum shock in rabbits. However, this dose is much larger than that which protects guinea pigs and dogs. The intramuscular injection of 40 mg./Kg. of Antergan in rabbits prevented anaphylaxis from the injection of horse serum. However, if the antigen was again injected eighteen to thirty-two hours later anaphylactic shock occurred. This suggests that antibody or antigen neutralization occurs only as long as the antihistamine is in the body and that there is no permanent neutralization (118).

Halpern (42) protected sensitized guinea pigs against a shock dose of cattle serum with 25–50 mg./Kg. of 2325 RP or 20 mg./Kg. of Antergan. Both compounds were able to prevent anaphylactic spasm of the sensitized guinea pig ileum *in vitro*. In horse serum sensitized dogs, 10 mg./Kg. of 2325 RP or 5 mg./Kg. Antergan prevented the depressor effect of 1 cc. of horse serum. The same protective

action was observed when Witte Peptone was used instead of horse serum.

Rose, *et al.* (145), showed that when equal, large doses (3 mg./Kg.) were administered to sensitized guinea pigs Neoantergan, Pyribenzamine, Antergan, and Benadryl were equivalent in protective power. However, when the doses were 0.1 mg./Kg. the drugs were rated as follows: Pyribenzamine, Antergan, Neoantergan, and Benadryl. The latter two compounds were equally effective in counteracting the antigen-induced spasm of the sensitized guinea-pig ileum. These results do not confirm the findings of Friedlaender, *et al.* (146), in so far as the lower dose is concerned but do confirm the equivalent potency of Pyribenzamine and Benadryl at the 3 mg./Kg. dose.

Lehmann and Young (44) compared equal doses (50 mg./Kg.) of diethylaminoethyl-9,10-dihydroanthracene carboxylate, diethylaminoethyl-xanthiene carboxylate and diethylaminofluorene-9-carboxylate in a large series of sensitized guinea pigs and found that the first compound gave 100% protection, the second 70%, and the last only 37%.

Rosenthal and Brown (78) showed that 0.5 mg. of thymoxyethyldiethylamine prevented the sensitized guinea-pig ileum from responding to the antigen *in vitro*. Similar results were obtained on the isolated guinea-pig uterus. *In vivo* experiments on guinea pigs showed that although all animals were protected against a dose of 0.04 mg./30 Gm. of antigen, larger doses caused a fatal reaction.

Staub and Bovet (105) have shown that 929 F protects guinea pigs from serum-induced anaphylactic shock. However, it does not protect sensitized rabbits against anaphylactic serum shock (115).

Reinhard and Scudi (133) found that 10 mg./Kg. intraperitoneally of both Neoheteramine and Pyribenzamine protected 60% of the sensitized guinea pigs against a shocking dose of horse serum. Benadryl protected only 30% at a dose of 25 mg./Kg.

Intraperitoneal injection of 2 mg./Kg. of Thephorin in sensitized guinea pigs protected 58% of the animals from fatal anaphylactic shock caused by the intracardial injection of 0.5 cc. of a 2% egg white solution (75).

Vallery-Radot, *et al.* (104), reported that 933 F and 883 F sometimes protected rabbits against anaphylactic shock. Rapid injection of 883 F prevents anaphylactic shock in rabbits (109).

Halpern (131) found that when sensitized guinea pigs received a shocking dose of serum 80% were protected by 0.1 mg./Kg. of 3277 RP, 60% by 2 mg./Kg. of 3015 RP, 67% by 0.5 mg./Kg. of Neoantergan, and 100% by 0.5 mg./Kg. of Antergan. Thus it would appear that the phenothiazine derivatives 3277 RP and 3015 RP are as potent as the older compounds in prevention of the fatal outcome due to anaphylactic shock.

Fatal anaphylactic effects caused by the intracardial injection of 1 cc. of horse serum in sensitized guinea pigs were prevented by protection with 3.5 mg./Kg. intraperitoneally of Heteramine (46).

Fifty mg./Kg. of 1135 F (*p*-hydroxyphenoxyethyldiethylamine) frequently but not always protected rabbits against fatal anaphylactic serum shock (129).

Experimental Skin Sensitization.—Dreisbach (99, 100) reported that neither Benadryl nor Pyribenzamine, 5 mg./Kg. subcutaneously twice daily, prevented the Arthus phenomenon in rabbits sensitized to penicillin or horse serum. Further, when these drugs were mixed with penicillin or horse serum they did not prevent a typical allergic response. However, when they were mixed with histamine under the same experimental conditions they did prevent the typical histamine wheal.

Benadryl does not sensitize or produce anaphylactic reactions in guinea pigs (50).

Mayer and Kull (134) found that pretreatment with Pyribenzamine almost completely suppressed the response of paraphenylenediamine-sensitized guinea pigs regardless of whether the antigen was injected with or without hyaluronidase.

Fischel (132) observed no modification in Arthus phenomenon induced in rabbits by the anti-egg albumin serum when Pyribenzamine was administered.

Mayer (142) showed that Pyribenzamine in doses of 10–25 mg./Kg. was effective in counteracting the urticarial action of hog serum in sensitized guinea pigs. However, after the effects of the drug had worn off the allergic response returned. In guinea pigs sensitized to paraphenylenediamine, Pyribenzamine and Antergan prevented the dermatitis seen in the unmedicated controls. Pyribenzamine was also effective in counteracting contact dermatitis in guinea pigs due to 2,4-dinitrochlorobenzene. Further work has shown that Pyribenzamine is effective in reducing the time of onset of the dermatitis caused by the local application of 10% mustard oil to guinea pigs (143).

Rennick, *et al.* (158), showed that wheal reaction to intradermal histamine in rabbits could be prevented by 1 mg./Kg. of Pyribenzamine intravenously or by 300 mg./Kg. orally. Further, capillary permeability as determined by the dye method is not increased.

Cullumbine (251) reported that Neoantergan subcutaneously, 10–40 mg./Kg. thirty minutes prior either to burning or injection of histamine or leucotaxine, prevented the spread of injected dye due to histamine but not to burning or leucotaxine.

Mixtures of Thephorin 1% and histamine 1:1000 to 1:10,000 when injected into the skin of the rabbit prevented the usual wheal formation and the spread of Trypan Blue seen when histamine alone was injected (51).

In the prevention of the spread of Trypan Blue in rabbits injected intracutaneously with histamine, the drugs used were rated as follows: Pyribenzamine, Neoheteramine, and Benadryl. Pyribenzamine was about five times more potent than either of the other drugs (137).

Last and Loew (144) showed that both Benadryl and Neoantergan reduced the spread of Trypan Blue usually seen after intradermal injections of histamine. Both drugs were effective when trypsin, tetracaine, and heparin were used instead of histamine but the responses were not significantly different from those of the controls. Benadryl produced no qualitative or quantitative differences in the spread of the dye when $MgSO_4$, horse serum, cobra venom, insulin, or staphylococcus toxin was injected.

Diethylaminoethyl - 9,10 - dihydroanthracene carboxylate intensified the intradermal reaction to histamine in rabbits when the drugs were injected together (44).

Boquet (108) found that 929 F, 883 F, and Anter-

gan had no effect on the intradermal reaction to tuberculin.

Respiratory Tract Effects.—Ellis and Newsome (124) tested the potency of a series of antihistaminic compounds related to Benadryl and found that tertiary amine derivatives such as Benadryl were more effective in counteracting histamine-induced bronchio-constriction in the perfused guinea-pig lung than quaternary ammonium derivatives. In the dog lung preparation *in situ* Benadryl was the most potent of all the compounds tested. The drugs were given in doses of 3 to 6 mg./Kg. after maximum constriction had developed due to the administration of 0.1 to 0.3 mg./Kg. of histamine. This confirms the previous observations of Ellis (156). However, Loew *et al.* (157) reported that conversion of Benadryl to a quaternary derivative increased its potency against the histamine bronchio-constriction.

Yonkman, *et al.* (102), found that Pyribenzamine, 25–50 μg., reduced bronchio-spasm induced by 50 μg. of histamine in the isolated guinea-pig lung. There was no marked effect on normal bronchial tonus. Depending upon the dose the relaxant effect lasted twenty to forty minutes and if Pyribenzamine was injected at the time of maximum constriction the protective effect of the drug was prolonged. In a similar series of experiments Antergan was as effective as Pyribenzamine against histamine spasm, but against the spasm caused by horse serum in sensitized lungs Pyribenzamine was not very effective. Pyribenzamine, 2 mg. intravenously, counteracted the spasmogenic effect of 15–50 μg. of histamine on the dog lung *in vivo*. The action was of long duration—three hours or more. Inasmuch as sensitized dogs did not exhibit a bronchial spasm upon injection of horse serum, Pyribenzamine could not be shown to be effective against this aspect of anaphylaxis.

Mayer, *et al.* (90), reported that 50 μg. of Pyribenzamine counteracted the bronchio-spasm induced by 50 μg. of histamine. This effect on the isolated guinea-pig lung was of forty to ninety minutes' duration.

Rennick, *et al.* (158), showed that 25–50 μg. of Pyribenzamine prevented bronchio-constriction usually produced by 50 μg. of histamine in the perfused guinea-pig lung. However, Pyribenzamine did not prevent bronchio-constriction in the horse serum sensitized lung upon addition of the antigen to the perfusion fluid.

Bronchio-spasm induced in guinea pigs by the intravenous injection of 1 mg./Kg. of histamine was prevented by the injection of 1 mg./Kg. of Antergan (42).

Sherrod, *et al.* (71), compared Neoantergan, Pyribenzamine, and Benadryl and found that the first two compounds were more potent in combating experimental bronchio-spasm in guinea pigs.

Lehmann and Young (44) found diethylaminoethyl-9,10-dihydroanthracene carboxylate in a dose of 1 mg. effectively produced bronchio-dilatation in the sensitized guinea-pig lung *in vitro*.

Lehmann, *et al.* (75), reported that 1 mg./Kg. of Thephorin prevented histamine-induced bronchio-spasm in cats.

929 F and 1571 F prevent the excitation provoked by histamine on the bronchioles (103).

Bronchio-constriction in the guinea pig due to histamine was prevented by premedication with 5 mg./Kg. of 929 F, 20 mg./Kg. of 1571 F, 1 mg./Kg. Antergan, or 0.5 mg./Kg. Neoantergan (113).

Injected Histamine.—Selle' (123) stated that 10 mg. of Benadryl protected guinea pigs against 3 *MLD* of histamine.

Wells and Morris (155) have stated that the amount of injected histamine inhibited bears a constant relationship to the amount of Benadryl injected within certain limits (1–16 mg./Kg.). Histamine is blocked at its site of action.

Against intravenous histamine, Pyribenzamine was more potent than Benadryl, Neoheteramine, or Heteramine. The second and third compounds were almost equivalent in potency (133).

Mayer, *et al.* (149), reported that when guinea pigs inhaled a 2% aerosol of Pyribenzamine they were protected against 7–15 *MLD* of intracardially injected histamine. Further, the duration of protection was relatively long.

Mayer and Brousseau (151) found that, even in doses of 25 mg./Kg. of Pyribenzamine or Benadryl, mice were not protected from the lethal effects of injected histamine; in fact, the toxic action of the latter was potentiated by both drugs.

Intraperitoneal or subcutaneous injection of 1 mg./Kg. of Thenylene protected guinea pigs against the shock induced by 0.4 mg./Kg. (LD_{100}) of histamine and 5 mg./Kg. of the antihistamine protected against 24 mg./Kg. of histamine (62).

Subcutaneous or intraperitoneal injection of 0.025 to 0.1 mg./Kg. of alpha-naphthylmethylethyl-β-chloromethylamine prevents or annuls the symptoms of shock in the guinea pig due to injected histamine. This antagonism is rapid in onset and markedly prolonged (64).

Halpern (42) found that 2325 RP in doses of 40 mg. protected guinea pigs against 50 fatal doses of histamine, whereas 10 mg. of Antergan counteracted 60 fatal doses of histamine.

Bovet, Horclois, and Walthert (43) reported that Neoantergan, 1 mg./Kg. subcutaneously, protected guinea pigs against 45 mg. of histamine intravenously (LD_{75}).

Lehmann and Knoefel (68) reported diethylaminoethyl-9,10-dihydroanthracene carboxylate (50 mg./Kg. subcutaneously) protected guinea pigs against 1.7 mg./Kg. of histamine phosphate intracardially.

Rosenthal and Brown (78) showed that thymoxyethyldiethylamine, 40 mg./Kg. subcutaneously, protected guinea pigs injected intracardially with 0.5 mg./Kg. of histamine.

Rose, *et al.* (145), rated the antihistamine drugs on their ability to protect guinea pigs from fatal histamine shock as follows: Neoantergan 125, Pyribenzamine 37, Antergan 6, and Benadryl 5. The numbers signified the number of LD_{100} of histamine counteracted by 3 mg./Kg. of antihistamine drug. Further, in equal doses Neoantergan was 4 times more potent than Benadryl. Friedlaender, *et al.* (146), rated the drugs as follows: Neoantergan 3.5 times more active than Pyribenzamine which is 6 to 7 times more active than either Antergan or Benadryl. This is in accord with their previous findings in which Pyribenzamine was found to be much more potent than Benadryl (147).

Bukantz and Dammin (159), using a method for measuring the fluorescence in the skin of rabbits, dogs, and humans after intravenous fluorescein, showed that intracutaneous histamine (1:10,000) could be blocked by Benadryl or Neoheteramine in a

concentration of 1:10,000 only in the last two species.

Thephorin, 5 mg./Kg. intraperitoneally, protected guinea pigs against 10–12 intracardial fatal doses of histamine and against death from anaphylactic shock (75).

Bromothen and Chlorothen were both three times more potent than Pyribenzamine in counteracting intravenous histamine in guinea pigs and, compared with Thenylene, Bromothen was twice as potent (54).

In the guinea pig, Halpern (131) found that 3277 RP counteracted 1500–1600 lethal doses of histamine while 3015 RP counteracted 400, Neoantergan 80, Antergan 50 to 60, and thymoxyethyldiethylamine 2 to 3. In rabbits 3277 RP counteracted 400–450 lethal doses of histamine, 3015 RP counteracted 100–125, Neoantergan 30, and Antergan 20–25. 3277 RP was not tested in the dog but the 3015 RP counteracted 2–25 lethal doses of histamine.

Staub (103) found that 929 F and 1571 F prevented histamine shock in guinea pigs. Staub and Bovet (105) had observed this effect with 929 F in 1937. Further, Vallery-Radot, *et al.* (104); observed this protective action in rabbits when 933 F and 883 F were administered. They also reported that rapid injection of 883 F immediately before the shocking dose prevents anaphylaxis (109). Vallery-Radot, Mauric, and Holtzer (115) found that 929 F strongly antagonized histamine shock in rabbits.

Premedication with 4, 5, and 6 mg./Kg. of Heteramine protected guinea pigs against the fatal histamine shock produced by the intracardial injection of 0.85 mg/Kg. of the latter (46).

Gastric Secretion.—Loew, *et al.* (59), found that Benadryl in a dose of 10 mg./Kg. subcutaneously to Heidenheim pouch dogs reduced gastric secretion in 3 of the 4 histamine-stimulated animals by 40%. One animal showed no reduction in secretion.

Sangster, Grossman, and Ivy (138) found that neither Benadryl nor Pyribenzamine (50 mg. subcutaneously) reduced the volume of secretion or the free acid output in four pouch dogs injected with histamine.

Friesen, Baronofsky, and Wangensteen (154) found that Benadryl had no effect upon the gastric secretory response of pouch dogs injected with histamine. Further, 100 mg. of Benadryl in beeswax mixture intramuscularly failed to protect dogs from histamine provoked ulcer.

Reuse (139) showed that Antergan, Neoantergan, 3277 RP, and Benadryl had no effect upon histamine induced gastric secretion in the guinea pig and cat.

Graham (94) reported that Neoantergan, Benadryl, or Antistine in doses of 4 mg./Kg. cutaneously in guinea pigs did not reduce the combined acid or abolish the free acid content of the ligated stomach. However, Neoantergan was the most potent in reducing the quantity of free acid induced by histamine.

Lehmann and Young (44), using dogs, showed that 20 mg./Kg. of diethylaminoethyl-9,10-dihydroanthracene carboxylate subcutaneously reduced the volume but not the acidity of histamine-induced gastric secretion. Lehmann and Knoefel (110) found that diethylaminoethylfluorene-9-carboxylate had a similar effect on meat extract stimulated gastric secretion. Both compounds were equal in potency in counteracting gastric motility induced by injection of 0.5 unit/Kg. of insulin in dogs.

Bourque and Loew (112) found that 1571 F had no effect upon food-stimulated gastric secretion in pouch dogs and pretreatment with 929 F did not modify gastric secretion in dogs injected with pilocarpine. Burchell and Varco (127) reported that thymoxyethyldiethylamine had no effect upon histamine-stimulated gastric secretion in Heidenheim pouch dogs.

Dreyer and Harwood (82) showed that 1–5 mg./Kg. of Neoheteramine did not reduce the total or free acid secretion in the cat when the left vagus was stimulated.

Halpern (131) was unable to show that the antihistamine drugs of the phenothiazine series (3015 RP, 3277 RP, etc.) had any effect upon gastric secretion in experimental animals. Further, these drugs although they were able to counteract histamine shock were unable to prevent histamine-induced ulceration and perforation of the stomach. This confirmed Halpern's previous observation (137) that neither 3015 RP nor 3277 RP prevented fatal histamine-induced stomach ulcer in guinea pigs although the other effects of the latter agent were blocked.

Histamine Asthma.—Intraperitoneal injection of 1.8 ±0.3 mg./Kg. of Benadryl protected 50% of the guinea pigs exposed to histamine aerosol (101).

Loew, Kaiser, and Moore (153) compared many drugs for their effect on histamine asthma in guinea pigs and rated them as follows: epinephrine, Benadryl, Demerol, atropine, papaverine, and aminophylline. Antispasmodics such as Trasentin, Pavatrine, and Syntropan which counteract acetylcholine were ineffective against histamine as were the local anesthetics, procaine and Nupercaine. Ergotamine tartrate likewise had no effect and physostigmine and ephedrine increased the severity of the asthma. It was concluded that a careful selection of drugs is necessary in order to obtain selective antispasmodic action on various smooth muscles.

Reinhard and Scudi (133) compared several antihistamines for their protective action against histamine asthma and rated them as follows: Pyribenzamine, Neoheteramine and Benadryl, and Heteramine. Further, when vaporized simultaneously with histamine, Pyribenzamine had more than four times the potency of Neoheteramine.

Lee, *et al.* (45), reported that the median protective dose of Thenylene was 0.152 ± 0.023 mg./Kg. as compared to Benadryl, 1.27 ± 0.36 mg./Kg. and Pyribenzamine, 0.059 ± 0.015 mg./Kg. when tested on guinea pigs in a histamine atmosphere. The concentration of histamine acid phosphate used was 0.187 μg./liter of air at a rate of 9 liters per minute.

Feinberg and Bernstein (194) found Thenylene more effective than Benadryl in counteracting histamine asthma in guinea pigs. However, Pyribenzamine was more potent than Thenylene under the same conditions.

Halpern (42) protected guinea pigs from the fatal effect of breathing atomized histamine for thirty minutes with 20 mg./Kg. of 1571 F, 10 mg./Kg. of 2325 RP, and 1.0 mg./Kg. of Antergan. Larger doses of the last two compounds protected animals for longer periods of time.

Neoantergan was four times more potent than Antistine and twice as potent as Benadryl when tested with histamine asthma in guinea pigs, and Benadryl was twice as potent as Antistine under the same conditions (94).

Wilcox and Siegal (128) found that 1571 F protected guinea pigs from fatal histamine and anaphylactic shock.

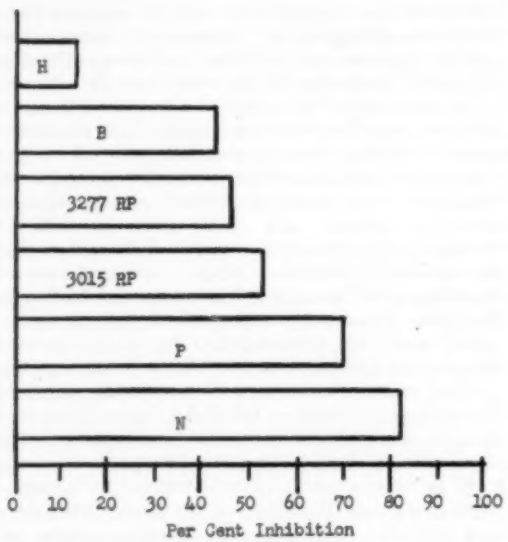
Lehmann, *et al.* (75), found that 0.5 mg./Kg. of Theophorin intraperitoneally protected 50% of the guinea pigs exposed to 0.4 mg. of histamine base per liter of air, while only 2% of the controls survived.

Litchfield, *et al.* (54), found both Chlorothen and Bromothen intraperitoneally three times more potent than Pyribenzamine in prevention of fatal histamine asthma in guinea pigs. Orally Bromothen was the most potent and when compared with Thenylene, Bromothen was about 3 times more potent.

Halpern (131) reported that 3277 RP was twice as effective in counteracting histamine asthma as Antergan and that 3015 RP was equivalent to the latter compound. Further, this action of 3277 RP had a duration of eight to nine hours, while that of Antergan and Neoantergan lasted only three to four hours. All the drugs were given at a dose of 1 mg./-Kg.

Feinstone, Williams, and Rubin (46) injected a dose of 0.3 to 2.5 mg./Kg. of Heteramine interperitoneally in guinea pigs and found that the drug significantly and increasingly reduced the mortality caused by breathing the vapor of 5 cc. of a 0.125% histamine solution.

Winter (160) made a comparison of the potency of six antihistamines and his results are given in Graphs I and II.



Graph I.—Inhibition of histamine-induced intestinal contraction in the guinea pig.
Equivalent doses of each drug were used.

H—Heteramine.
B—Benadryl.
P—Pyribenzamine.
N—Neoantergan.

Miscellaneous.—Kyser, McCarter, and Stengle (125) found that the Benadryl, 10 mg./Kg. subcutaneously twice daily, was effective in preventing serum-induced myocarditis in rabbits. When the dose was decreased to 2 mg./Kg. the drug was only partially effective.

Traub, *et al.* (148), reported that 7.5 mg./Kg. of Benadryl suppressed the action of 1:10,000 histamine on the skin capillaries of the rabbits by 75% to 100%.

Arbesman, *et al.* (227), found that Pyribenzamine had no effect on the precipitin titer or guinea-pig complement titer.

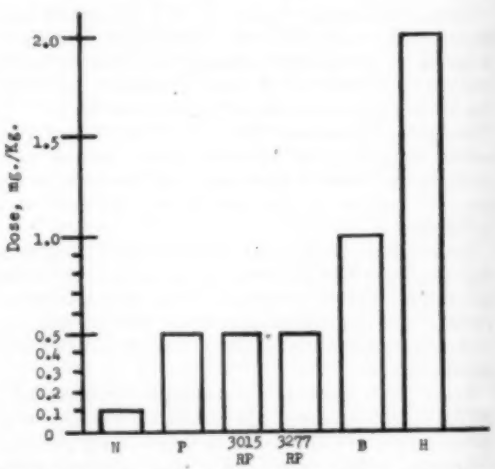
Mayer and Kull (134) showed that Pyribenzamine and Antistine in equal doses had an equivalent effect in the prevention of the spread of India ink injected intradermally in rats which had been injected with hyaluronidase.

Mayer, Eisman, and Aronson (150) reported that Pyribenzamine has no influence upon the immunization activities of the body or on the chemotherapeutic activity of antibacterial agents.

Boquet (114) showed that the Sanarelli-Schwartzman phenomenon induced by injection of *E. coli* filtrates was not affected by 929 F or Antergan. Antergan also decreases the mortality of guinea pigs exposed to freezing by CO_2 snow (119). Di Macco (120) showed that histamine increased the volume of peritoneal exudate in the guinea pig induced by croton oil and Antergan decreased it.

Reuse (139) reported that Antergan, Neoantergan, 3277 RP, and Benadryl all counteracted histamine-induced spasm of the longitudinal muscles of the stomach of the guinea pig and of the cat.

Antergan, Neoantergan, Benadryl, and 3277 RP decreased the effect of histamine on the isolated perfused cat heart, but the drugs were not as effective in combating this effect of histamine as they were



Graph II.—Dose required to protect most of the animals against lethal histamine aerosol.

N—Neoantergan.
P—Pyribenzamine.
B—Benadryl.
H—Heteramine.

upon isolated smooth muscle. Neoantergan had a potency three times that of Benadryl by this test. On the isolated rabbit heart Neoantergan was more potent than 3277 RP, Antistine, or Benadryl in counteracting the effects of histamine (130).

Capraro (121) reported that Antergan and Benadryl have a dilating action on large arteries, making them insensitive to the action of injected histamine. However, Benadryl has no effect on the

capillaries so that capillary dilatation due to histamine occurs even after Benadryl.

Neoantergan, 25 μg., reduced the dilatating effect of 25 μg. of histamine on the perfused cat heart coronary vessels and on the coronary vessels of the dog heart-lung preparation. Further, the drug abolished the stimulant effect of histamine on the isolated rabbit auricles. Upon the driven rabbit auricles, Neoantergan was twice as active as quinidine in reducing the rate of contraction (97).

Reinhard and Scudi (133) found that Neoheteramine and Pyribenzamine were equal in combating histamine spasm of the excised guinea-pig tracheal chain. Castillo and de Beer (76, 77) found Benadryl also counteracted the histamine spasm of the tracheal chain.

Effect on the Isolated Intestine.—The spasmogenic effect of histamine on the intact dog duodenum was counteracted by Benadryl, Pyribenzamine, and Neoantergan (71).

Winder, *et al.* (87), found that 1.1 mg. of Benadryl/100 cc. of solution reduced the spasm elicited by 8 μg. of histamine by 68%.

Selle (123) found that 0.1 mg. of Benadryl counteracted the spasmogenic effect of 10 μg. of histamine on the guinea-pig ileum. Upon the sensitized guinea-pig ileum 0.2 mg. of Benadryl was required to counteract the assault dose of antigen.

Pyribenzamine, 0.01–0.02 μg., usually counteracted the spasm produced in the isolated guinea-pig jejunum by 1 μg. of histamine (102).

Hoekstra and Steggerda (72) showed that a dose of 0.5 mg./Kg. of Pyribenzamine did not counteract the effect of 10–30 μg. of histamine on the dog colon *in situ*. However, 1.5–2.5 mg. of the antihistamine was effective in counteracting histamine and the duration of activity was up to fifty minutes.

Cameron and Craver (126), using dogs with Thiry-Vella loops, found that after the administration of antihistaminic drugs, histamine often gave a significant inhibition of motility which might or might not be followed by a secondary stimulation. This action was probably due to stimulation of the sympathetic ganglia by histamine because adrenalectomized animals showed that epinephrine was not the causative agent.

Lee, *et al.* (45), reported that Thenylene, weight for weight, was 4.9 times as active as Benadryl and 0.8 times as active as Pyribenzamine when tested by its relaxant effect on histamine-induced spasm of the isolated guinea-pig ileum. The spasm of the isolated guinea-pig ileum caused by 0.005 μg./cc. of histamine can be inhibited at least 75% or relaxed by 0.002 μg./cc. of Thenylene (62).

Halpern and Mauric (107) found that there was a definite quantitative relationship between the amount of histamine which was antagonized by Antergan when tested with the isolated guinea-pig ileum.

Halpern (42) found that the spasm of guinea-pig intestine induced by histamine could be abolished by 15 μg. of 1571 F, 1.5 μg. of 2325 RP, and 0.1 μg. of Antergan.

Reuse (140) showed that Antergan was effective in preventing serum-induced contraction of the sensitized guinea-pig ileum *in vitro*. In his later work, Reuse (141) found that Antergan, Neoantergan, and 3277 RP all were able to counteract the intestinal spasm of the isolated guinea-pig ileum produced by the H-substance liberated during anaphylactic shock.

Neoantergan (0.1 μg.) reduced the response of the isolated guinea-pig ileum to 1 μg. of histamine by more than half its previous level (97).

Graham (94) found that Neoantergan, Benadryl, and Antistine were rated in that order when tested upon the isolated guinea-pig ileum.

Reuse (130) found that Neoantergan and 3277 RP were much stronger than Antistine in preventing histamine-induced spasm of the guinea-pig ileum *in vitro*. It was also shown that the duration of action and the amount of inhibition observed were proportional to the dose of the drug. Halpern (131) reported that 3277 RP, 3015 RP, and Antergan were equal in antagonizing the effect of histamine on the isolated guinea-pig ileum.

Using a comparative method, Schild (136) found Neoantergan more potent than Benadryl in counteracting histamine spasm of the guinea-pig ileum *in vitro*. This antihistamine effect of Neoantergan was greater the longer the drug remained in contact with the tissue.

Lehmann and Young (44) found diethylaminoethyl-9,10-dihydroanthracene carboxylate 3 times stronger than 1571 F when tested upon the isolated guinea-pig ileum. Lehmann and Knoefel (68, 69) showed that diethylaminoethyl-fluorene-9-carboxylate and diethylaminoethyl-xanthene-9-carboxylate were both less potent than the anthracene compound.

Thymoxyethyldiethylamine (929 F) strongly inhibited histamine-induced intestinal spasm (127).

Rosenthal and Brown (78) found that 0.5 μg. of thymoxyethyldiethylamine prevented the intestinal spasm usually produced by 0.02–0.04 μg. of histamine on the isolated guinea-pig ileum.

Although Neoheteramine was active in abolishing histamine-induced spasm of the isolated guinea-pig ileum it was not so effective on this as on other smooth muscles (82).

Lehmann, *et al.* (75), found that 0.01 mg./cc. of Thephorin blocked the effect of 0.2 mg. of histamine phosphate. The effect increased as the interval between the administration of the two drugs increased.

Chlorothen and Bromothen are equivalent in potency and 4 times more active than Pyribenzamine in suppressing histamine-induced spasm of the guinea-pig ileum. Further, Bromothen is more than 3 times as active as Thenylene by the same test (54).

929 F and 1571 F are antagonistic to the intestinal spasm induced by histamine (103).

Ungar, Parrot, and Bovet (106) antagonized the spasmogenic effect of histamine with 933 F.

Heteramine, 1 μg./cc. of bathing fluid, prevented the contraction of the isolated guinea-pig ileum usually produced by 1 μg./cc. histamine (46).

Effect on the Uterus.—Selle (123) reported that the effect of 10 μg. of histamine on the isolated guinea pig uterus was prevented by 0.1 mg. of Benadryl.

Sherrod, *et al.* (71), found that Benadryl, Pyribenzamine, and Neoantergan all given intravenously in a 3 mg./Kg. dose counteracted the spasmogenic effect of injected histamine on the dog uterus.

Contraction of the isolated guinea-pig uterus produced by 10–20 μg. of histamine was counteracted by 10–50 μg. of Pyribenzamine (90).

Çayet-Hallion and Quiyy (116) reported that

Antergan inhibited the action of histamine on the isolated guinea-pig uterus but this inhibition was abolished by doubling the calcium or magnesium-ion concentration of the bathing fluid.

Dews and Graham (97) found that 0.2 μg. of Neoantergan significantly reduced the response of the isolated guinea-pig uterus to 3 μg. of histamine. They confirmed the results of Bovet and Walthert (92) on the isolated rat uterus, finding that Neoantergan relaxed this organ in the same manner that histamine did. Thienes (111) has observed this same effect with Benadryl and Pyribenzamine.

Reuse (130) has shown that Neoantergan was more potent than either 3277 RP or Benadryl in suppressing the *in vitro* response of the nonpregnant cat uterus to histamine. 3277 RP had to be added to the bath one to two minutes prior to the addition of histamine and even then it was more effective in blocking the second rather than the first dose of the latter. No difference was seen in the response of the pregnant or nonpregnant rabbit uterus to Neoantergan, 3277 RP, Benadryl, or Antistine after histamine. However, the first compound was more potent than the others in its blocking action. Both Neoantergan and 3277 RP had a similar action on the nonpregnant guinea-pig uterus. Halpern (131) had previously observed that 3277 RP had a histamine blocking effect on the isolated guinea-pig uterus.

Lehmann and Young (44) showed that diethylaminoethyl-9,10-dihydroanthracene carboxylate was more potent than diethylaminoethyl-xanthene carboxylate and diethylaminoethyl-fluorene-9-carboxylate in preventing the response of the sensitized guinea-pig uterus to antigen.

Dreyer and Harwood (82) showed that Neoheteramine counteracted the histamine spasm of the isolated cat and guinea-pig uterus and was more effective on the former. Neoheteramine relaxed the isolated rat uterus similarly to Benadryl and Pyribenzamine.

Staub (103) reported that 929 F and 1571 F were effective in preventing histamine spasm of the isolated uterus.

CLINICAL USAGE

Human Pharmacology.—McGavack, Elias, and Boyd (161, 162) reported that Benadryl in the usual doses had no effect upon the body weight or BMR of humans. Upon the central nervous system the drug has a sedative effect. Orally Benadryl had no effect on the eye but local application of a 0.5% solution caused moderate dilatation while a 1% solution produced mydriasis with an interference with accommodation. Doses of 400 mg. returned the respiratory rate and vital capacity of asthmatics to normal. In doses of 300 mg. in normal humans there were no changes in the lungs, pulse rate, heart rate, nail bed capillary loop, electrocardiogram, but in some patients the systolic blood pressure was decreased. Body temperature was unchanged with oral doses of the drug but intravenous administration decreased the body temperature 1° F. The volume of urine and its constituents were not changed nor was there any interference with the concentration or dilution tests. Urea nitrogen and blood NPN were unchanged in the usual doses. There was no change in the hematological constituents or the blood chemistry after Benadryl. Circulation time was unchanged and capillary permeability as measured by the dye method decreased. The gastrointestinal tract was not disturbed in regard to function because the mouth-anus time was unchanged. There was no increase in salivary secretion but the drug did increase gastric acidity in younger patients while it decreased it in the elderly even after histamine stimulation. The oral administration of Benadryl (50–100 mg.) causes a definite decrease in blood pressure. This decrease may be of two or more hours' duration after a single dose (182). Reinstein and McGavack (163) have shown that 400 mg. of Benadryl orally had no effect upon the glucose tolerance curves in young or elderly humans but that 30 mg. of the drug intravenously increased glucose tolerance.

Koepf, *et al.* (95), reported that 3 normal male subjects 22–24 years of age showed no changes in body weight, hematology, blood chemistry, blood pressure, or liver function after receiving 150 mg. of Pyribenzamine orally for eighty days.

Gastric Secretion.—The antihistamine drugs apparently have little or no effect upon histamine-stimulated gastric secretion in man. McGavack, Elias, and Boyd (164) gave 200, 300, and 400 mg. of Benadryl orally daily in divided doses for periods of from two to seven weeks. Upon the basis of predetermined normal gastric acidity levels following the administration of 50 cc. of a 7% alcohol meal, they concluded that the drug had a depressant effect upon gastric acidity. Both free and total acid secretion were depressed but no gastrointestinal symptoms were observed in the 8 normal subjects and the 5 patients with gastrointestinal neurosis.

Moersch, Rivers, and Morlock (165) reported that there was no reduction in the concentration of free hydrochloric acid and no consistently significant reduction in the total volume of secretion following intravenous Benadryl in doses of 60, 100, or 200 mg. Fifteen cases of proved and one case of suspected duodenal ulcer were used as test subjects. The histamine was administered either subcutaneously or intravenously. In 3 patients an arrowroot cookie test meal was used as gastric secretory stimulator rather than histamine. Regardless of the stimulator used the results were the same.

McElin and Horton (166) investigated the histamine-induced rise in gastric acidity in 8 subjects with multiple sclerosis. Three of their patients who received the drug intravenously (40–60 mg.) gave results which suggested that Benadryl inhibited the histamine-induced rise in acidity. Similar results were obtained with one patient who was premedicated orally (400–620 mg.) fourteen hours before the test or intramuscularly (20 mg.). One patient did not respond to histamine and one had a Benadryl-provoked rise in gastric acidity. Two other patients showed no inhibition of gastric acidity by Benadryl regardless of the mode of administration.

Wolf and Wolff (168) reported that Benadryl (50 mg.) increased mucin secretion, viscosity of the secretion, slightly decreased the color, and decreased the total acidity and turgidity of gastric secretion during the phase of average gastric function. However, the drug had no effect during a phase of gastric hyperactivity related to emotional stress. The first effect may have been due to the slowly induced but long acting local vasoconstriction caused by Benadryl.

Serafini and Biozzi (169) found that 2 cc. of a 2% solution of Antergan administered after subcutaneous injection of 1 mg. of histamine HCl in 10 patients had no effect on the hypersecretion of gastric juice.

Decourt (170) found that Neoantergan had no effect on the hydrochloric acid content of gastric juice, or on the gastric response to histamine.

Miscellaneous Effects.—Leavitt and Code (171) tested the anesthetic effect of Benadryl injected intradermally in 10 humans. One forearm was injected with 0.3 cc. of Benadryl (1:500, 1:1000, 1:5000, 1:10,000, and 1:20,000) while the other was injected with procaine (1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200). Anesthesia was tested using electric stimulation from an algesimeter. Benadryl was shown to possess definite local anesthetic properties but when injected at concentrations greater than 1:500 it produced tissue necrosis and ulceration in 4 of the 10 subjects tested.

Gelvin and McGavack (38) showed that the blood and spinal fluid concentrations of Benadryl were approximately equal after the administration of oral doses of 100–400 mg. to six human subjects. Gelvin, Elias, and McGavack (172) explain this upon the basis of the unaltered permeability of the meningeal capillaries. The five patients studied received 1900 to 3450 mg. of Benadryl during one week. No changes in the spinal fluid were recognized nor did the disturbances in activity of the central nervous system cause any alteration in the formation or character of the fluid.

Pellerat and Murat (173) showed that subcutaneous injections of either Antergan or Neoantergan caused a 6- to 12-fold increase in the blood histamine levels for more than twenty-four hours. Similar results were obtained by Staub (174) when 0.2 mg. of epinephrine was injected intravenously. However, the intravenous injection of 200 mg. of Antistine prior to the epinephrine prevented or diminished the blood histamine rise.

Acute Urticaria.—Benadryl, in dosage fitted to the needs of the patient, has proved very effective in the treatment of acute urticaria (175, 182–185). In general, the pruritis associated with this condition is relieved within twenty to sixty minutes after the first 50–100-mg. dose of the drug and all lesions disappear within two to six hours (178). Doses of 2–20 mg. two to four times are effective in children (180). Benadryl is effective in the treatment of drug rash (185). Relief of the urticaria and partial relief of the angioneurotic edema caused by insulin sensitivity by the use of 250–350 mg. of Benadryl has been reported by Gastineau and Leavitt (180, 181). Further, Benadryl at various dilutions reduced the flare reaction caused by the intracutaneous injection of 0.2 cc. of insulin.

Benadryl and Pyribenzamine are about equal in the relief of the urticaria, particularly that caused by drug sensitivity (195).

Rauchwerger, *et al.* (198), found that 50 mg. of Benadryl four times daily for three days had no effect on acute urticaria due to streptomycin. However, 50 mg. of Pyribenzamine four times daily gave relief within twelve hours in three patients.

Pyribenzamine, in dosage of 50–500 mg. daily, gives definite relief in acute urticaria, but the drug is only a palliative (188, 188–190, 253).

Goodhill (191) relieved 5 cases of penicillin urticaria with 50 mg. of Pyribenzamine every four to six hours.

Thenylene is beneficial in controlling the pruritis of acute urticaria (194). Comparison of this drug with Benadryl and Pyribenzamine shows it to be equally effective but at a somewhat larger dose (254).

Sixteen of 17 cases of drug and serum reactions were relieved by Thenylene 150–300 mg. daily. These cases included patients sensitive to Thiouracil, Penicillin, streptomycin, and horse serum (195).

Neoantergan is very effective in relieving acute urticaria (176). Hunter controlled the acute urticaria due to penicillin in 5 cases with 300–800 mg. of Neoantergan daily. In 2 cases penicillin was not discontinued while Neoantergan was being given but no rash recurred. Hunter and Hill (193) controlled the urticarial reaction of 8 of 9 patients to liver extract with 200–800 mg. of Neoantergan daily. Reactions to zinc protamine or globin insulin were also controlled by the drug.

Ereaux and Craig (197) reported Anthallan gave prompt relief in drug rashes due to sulfathiazole and penicillin.

Thephorin relieved the itching and urticaria due to both cold sensitivity and food (255).

Antistine produces symptomatic relief for periods of two to twelve hours in acute urticaria. Comparison with Benadryl and Pyribenzamine showed that Antistine was equally effective in treating this condition (256).

Table V gives a comparison of the effectiveness of several antihistamines in acute urticaria.

TABLE V.—EFFECTIVENESS OF ANTIHISTAMINES IN ACUTE URTICARIA

Drug	Daily Dosage, Mg.	Total Number of Cases	Total Number of Cases Relieved
Benadryl	50–350	71	61
Pyribenzamine	50–400	193	157
Thenylene	50–300	12	7
Neoantergan	50–400	13	11
Thephorin	50–350	11	7
Antistine	50–400	10	7

Vasomotor Rhinitis or Hayfever.—This condition, seasonal or nonseasonal, may be controlled with Benadryl but unless the drug is continued the symptoms recur. Relief usually begins within thirty-five to sixty minutes and is of varying duration, often requiring dosage at two-hour intervals (175, 179, 190, 199–204, 210). The drug has been shown to be effective in the treatment of vasomotor rhinitis in children (202). Further, it is more effective in children (2 of 3 cases) than in adults (2 of 11 cases) (179). It has been reported that vasomotor rhinitis is not ameliorated by Benadryl (182).

Pyribenzamine has been used successfully in the treatment of nonseasonal allergic rhinitis (187–189) and in chronic allergic rhinitis (188). Goodhill (191) noted that there was a reduction in the excessive rhinorrhea, a wider airway, and the sneezing, palatal pruritis, and edema ceased or were greatly reduced in patients with acute or chronic allergic rhinitis after Pyribenzamine.

Thenylene gives symptomatic relief in hayfever (195) and some measure of relief in nonseasonal vasomotor rhinitis (194).

Bernstein, Rose, and Feinberg (195) compared Neoantergan, Benadryl, and Pyribenzamine by their effects in patients with hayfever. All the drugs were effective but inasmuch as the total number of cases tested with each drug were not equal it is hard to designate the most effective of the three. Further, some patients responded to one drug but not to the others, showing that the clinician should utilize the drug which gives the desired results regardless of the animal experiments which show that one drug is more potent than another. Further, Criep (206) recently stated that Neoantergan, Thenylene, Benadryl, and Pyribenzamine produced only a palliative relief of vasomotor rhinitis.

Westcott (205) treated perennial vasomotor rhinitis with both Anthallan and Benadryl and found the former compound only 20% effective. Ghiselin (215) reported that Anthallan was effective in relieving seasonal and nonseasonal hayfever.

Antistine also produces symptomatic relief in patients suffering with allergic rhinitis. Further, this drug compares favorably with Pyribenzamine in relieving this condition (256).

Table VI gives a comparison of the effectiveness of several antihistamines in vasomotor rhinitis.

TABLE VI.—EFFECTIVENESS OF ANTIHISTAMINES IN VASOMOTOR RHINITIS

Drug	Daily Dosage, Mg.	Total Number of Cases	Total Number of Cases Relieved
Benadryl	50–300	502	420
Pyribenzamine	50–300	1136	831
Thenylene	100–400	229	141
Neoantergan	50–1500	121	78
Anthallan	85–340	42	42
Antistine	50–400	59	35

Chronic Urticaria.—Benadryl is beneficial in the treatment of this condition but the drug is only a palliative as the itching and wheals recur when the drug is stopped (175, 177, 179, 182, 183, 185, 199, 201, 204, 207). Intravenous injection affords relief when oral medication is ineffective (208). Logan (202) controlled chronic urticaria in children with Benadryl, 2 mg./lb. body weight.

Benadryl was more effective than Pyribenzamine in the treatment of urticaria due to penicillin. Dosage of either drug was 50–100 mg. three times daily (207).

Chronic urticaria responds to Pyribenzamine therapy but continuous medication is required to prevent recurrence of the condition (186–188, 190, 253).

Thenylene has only been tested in a small number of cases but the drug is as effective as other antihistamines in current use (196, 254).

Hunter (192) controlled chronic urticaria associated with angioneurotic edema with Neoantergan. No tolerance developed in three months and the urticaria recurred within ten hours to four days after the drug was stopped.

Schwarzschild (209) reported that Anthallan relieved chronic urticaria and papular urticaria.

Kesten and Sheard (255) found that Thephorin relieved chronic urticaria within a few hours after the first 25–50 mg. dose. Continuous medication did not produce tolerance and the attacks were shorter, less severe, and the itching usually absent.

Friedlaender and Friedlaender (256) showed that although Antistine was only 10% as active as Pyribenzamine by the guinea-pig test, it was fully as effective clinically. However, the effective dose of the latter is twice that of the former.

Table VII gives a comparison of the effectiveness of several antihistamines in chronic urticaria.

TABLE VII.—EFFECTIVENESS OF ANTIHISTAMINES IN CHRONIC URTICARIA

Drug	Daily Dosage, Mg.	Total Number of Cases	Total Number of Cases Relieved
Benadryl	50–500	221	181
Pyribenzamine	50–400	241	138
Thenylene	50–400	18	15
Neoantergan	300–800	8	8
Anthallan	85–340	49	45
Thephorin	100–300	15	15
Antistine	200–400	9	3

Asthma.—Benadryl may or may not be beneficial in bronchial asthma and in general such drugs as ephedrine are more effective (199). Where asthma is associated with hayfever or pollinosis Benadryl has proved to be helpful (175, 179, 182, 201, 202, 204, 210, 240).

Levy and Seabury (212) reported that after the oral administration of 100 mg. of Benadryl there were no consistent changes in vital capacity, tidal air, minute ventilation, expiratory differential, respiratory rate, or degree of emphysema in 16 asthmatics. These tests were performed with a spirometer thirty to sixty minutes after administration of the drug. Curry (213) demonstrated that 10–30 mg. of Benadryl intravenously reduced the bronchio-constrictor effect of 0.02 mg. of histamine intravenously in 2 test subjects. The vital capacity reduction after Benadryl was less than half that produced by histamine alone. Pyribenzamine, 50 mg. orally, had a delayed onset of action in 3 patients, but gave results similar to Benadryl. Systemic effects (flushing, headache, and taste) were partially blocked in all cases.

Epstein (190) reported that asthma was relieved when either Benadryl or Pyribenzamine was given in equal doses. However, in general, results are disappointing. Goodhill (191) had similar results with asthmatics.

Pyribenzamine is no more effective in asthma than Benadryl (187–189, 253). Koepf, *et al.* (214), reported that Pyribenzamine and ephedrine were more effective than either drug alone in the treatment of bronchial asthma.

Feinberg and Bernstein (194) found that Thenylene did not decidedly help the dyspnea of asthma in 30 patients although it did help the preasthmatic-spasmodic cough in 6 of 9 patients. Thenylene is of little benefit in bronchial asthma even in doses of 400 mg. daily (195).

Neoantergan is not very effective in relieving either bronchial asthma or pollen asthma (176). Bernstein, Rose, and Feinberg (195) reported that the incidence of benefit and the degree of relief were only moderate in asthmatics given Neoantergan, Benadryl, or Pyribenzamine. Children generally responded more favorably than adults and the effect was greater on the cough than on the dyspnea.

Antistine benefits the cough to a greater degree than the dyspnea in asthmatics. Where asthma is associated with rhinitis, the latter is benefited even when the asthmatic condition is not. Antistine is as effective as Pyribenzamine in the treatment of asthma, but the results with both drugs are disappointing (256).

Table VIII gives a comparison of the effectiveness of several antihistamines in bronchial asthma.

TABLE VIII.—EFFECTIVENESS OF ANTIHISTAMINES IN BRONCHIAL ASTHMA

Drug	Daily Dosage, Mg.	Total Number of Cases	Total Number of Cases Relieved
Benadryl	50–400	213	101
Pyribenzamine	100–400	327	109
Thenylene	100–400	30	0
Neoantergan	100–400	18	7
Antistine	200–400	24	9

Atopic Dermatitis.—Benadryl benefits pruritis more than atopic dermatitis (179, 182–184, 204).

Epstein (190, 216) found both Benadryl and Pyribenzamine effective in controlling the pruritis associated with atopic dermatitis and drug eruptions in elderly patients. However, the latter was more effective in atopic dermatitis. Bernstein, *et al.* (195), found Benadryl and Pyribenzamine about equal in relieving the itching of atopic dermatitis. The lesions were not consistently benefited by either drug but with a decrease in itching some improvement was noted in the lesions.

Perry (218) reported that topical application of 2% Benadryl ointment relieved only 8 of 22 patients suffering from various pruritis dermatites. However, poor absorption of the ointment was probably the cause of these results.

Pyribenzamine gives relief to the patient suffering from atopic dermatitis (185). Pyribenzamine ointment relieved the itching of atopic dermatitis in 24 of 33 cases (217).

Peirce and Mothersill (196) found that Thenylene was not too effective in counteracting pruritis. However, it has been stated that Thenylene relieves the itching of atopic dermatitis. Thenylene is as effective as either Benadryl or Pyribenzamine in relieving the pruritis associated with atopic dermatitis (254).

In the small number of cases thus far tested, Neoantergan has not been too successful in relieving atopic dermatitis. However, it does relieve pruritis (176).

Schwarzschild (209) found Anthallan gave relief of varying degrees in atopic dermatitis (*neurodermatitis disseminata*). Ereaux and Craig (197) were able to relieve this condition by giving 85 mg. of Anthallan 3 to 4 times daily. Infantile eczema was also benefited.

Thephorin is remarkably effective in allaying itching of atopic dermatitis (255).

Antistine relieves atopic dermatitis and is as effective as Pyribenzamine when tested in the same patient. In some cases one drug was effective after the other had failed to give relief (256).

Table IX gives a comparison of the effectiveness of several antihistamines in atopic dermatitis.

TABLE IX. EFFECTIVENESS OF ANTIHISTAMINES IN ATOPIC DERMATITIS

Drug	Daily Dosage Mg.	Total Number of Cases	Total Number of Cases Relieved
Benadryl	50–150	45	23
Pyribenzamine	50–150	44	30
Thenylene	50–200	19	11
Neoantergan	50–400	14	3
Anthallan	85–340	59	51
Thephorin	150–300	15	7
Antistine	50–400	5	3

Other Conditions.—Goodman and Coonrad (222) have found Benadryl, 100 mg., very effective in the prevention of histamine-induced headache. In 18 patients known to develop headache after histamine injections, Benadryl prevented the condition in fifteen.

Brewster (223) has stated that Benadryl alleviates or abolishes the symptoms of the common cold in both adults and children. A 50-mg. capsule of the drug was given at the first sign of nasopharyngeal soreness and serious discharges were eliminated and the cough reflex inhibited. However, in view of the small number of cases treated, Benadryl should not be considered as a specific in the treatment of the common cold.

Logan (202) relieved a 3-year-old child, suffering from nephrosis, with Benadryl. Schwartz and Levin (175) employed Benadryl, 50 mg. per dose, as a palliative in the treatment of allergies of miscellaneous origin. Hypersensitiveness to cold was reduced by the oral administration of 200 mg. of Benadryl daily for forty-eight days. Regression occurred when the drug was discontinued (219). Benadryl was effective in relieving 2 of 3 cases of cold urticaria and one case of heat urticaria. It also was effective against penicillin reaction in 3 patients (182). Feinberg and Friedlaender (220) tried Benadryl in several cases of rhinitis and asthma which were precipitated by temperature changes. Their results were negative. Benadryl, 200–300 mg. daily, gave relief to 4 patients with scleroderma and to 9 patients with acrosclerosis (183, 184). Lynch (185) found Benadryl effective in the treatment of pruritis vulvae, insect bites, and erythema multiforme. Lofstrom and Nurnberger (224) found that 50–150 mg. of Benadryl intravenously or 50–200 mg. orally counteracted the symptoms of radiation sickness. Epstein (190) successfully treated pruritis ani and vulvae and drug rash with Benadryl and Pyribenzamine 50 mg. three or four times daily.

Baer and Sulzberger (186) relieved 13 of 28 cases of pruritis associated with miscellaneous dermatoses by the oral administration of 50–300 mg. of Pyribenzamine daily. Pyribenzamine, 50–100 mg. per dose, prevented hypersensitiveness to cold (187). Feinberg and Friedlaender (188) found Pyribenzamine of benefit in unclassified dermatitis, drug rash, penicillin allergy, chronic headache, gastrointestinal allergy, and pruritis vulvae. However, even at a dose of 100 mg. four times daily the drug was only a palliative. Penicillin urticaria in a syphilitic was controlled by Pyribenzamine 40 mg. every two hours (221). Topical application of 2% Pyribenzamine ointment relieved the itching in 3 cases of dermatitis of unknown origin and 8 cases of pruritis ani (217). Fischel (132) reported that 25–50 mg. of Pyribenza-

mine daily had no effect on the Arthus reaction induced by purified bacterial antigens injected into adults and children.

Peirce and Mothersill (196) reported that 200 mg. of Thenylene daily relieved sensitivity to cold, had no effect in hypertensive headache or histamine-induced headache, but gave moderate relief in functional headache. Thenylene was as effective as either Benadryl or Pyribenzamine in the treatment of anal and vulval pruritis (254).

Neoantergan, 50–400 mg. three times daily, has not been found to be beneficial in the treatment of angioneurotic edema, fungus infection of the skin, gold dermatitis, poison ivy dermatitis, or vernal conjunctivitis. However, when more cases have been treated this may prove to be incorrect (176).

Thephorin, 150–300 mg. daily, relieves erythema multiforme, papular urticaria, neurodermite, and pruritis ani, but not psoriasis. Hypersensitiveness to cold was also relieved (255).

Antistine, 200–400 mg. daily, was not found to be beneficial in pruritis ani or dermatitis of unknown origin. However, more cases must be investigated before any conclusions can be drawn (256).

Contact Dermatitis.—Levin (179) found Benadryl partially effective against contact dermatitis and drug rash. Friedlaender and Feinberg (182) found that only the pruritis associated with this condition was controlled with Benadryl.

Feinberg and Bernstein (217) relieved two cases of contact dermatitis with Benadryl and Pyribenzamine.

Peirce and Mothersill (196) successfully relieved 3 of 4 cases of contact dermatitis with 200 mg. of Thenylene daily. Neurodermatitis was also slightly benefited.

Histamine Flare Reaction.—Friedlaender and Feinberg (182) reported only a slight inhibition to intradermal histamine (1:1000–1:100,000) after oral administration of 50 mg. of Benadryl or Pyribenzamine. A similar inhibition is also produced against ragweed, mechanical, or chemical whealing. A marked reduction was obtained when the antihistamine was applied locally to the injection site. Elias and McGavack (225) found that 300–400 mg. of Benadryl daily reduced the erythema and wheal reaction caused by the direct application of one drop of 1% histamine to the volar surface of the forearm. Premedication with Benadryl enabled Feinberg and Friedlaender (220) to perform scratch tests for sensitivity to various substances because the drug suppressed the dermographic response previously obscuring the results. The dosage was regulated to fit the needs of the 20 adults and 10 children tested.

Grob, Lilienthal, and Harvey (226) found that the histamine flare and wheal reaction produced by the intracutaneous injection of 0.05 cc. of curare or *d*-tubocurarine was inhibited by prior injection of 150 μg. of Pyribenzamine or 1.5 mg. of Benadryl. Mixing of the drugs before injection gave the same results, but the effect was less when 0.1 mg. of histamine was used instead of curare or *d*-tubocurarine. Intra-arterial injection of 10 mg. of Pyribenzamine reduced the hyperemia, venous engorgement, and ecchymotic discoloration produced by the intra-arterial injection of 50 units of curare. The usual temperature rise associated with curare injections was also inhibited. Oral administration of 200 mg. of Pyribenzamine at one hundred and thirty minutes prior to the injection of 50 units of curare had the same effect.

Arbesman, Koepf, and Miller (227) observed a definite reduction in the histamine wheal and flare produced by both serial dilutions of histamine and allergens after 50–150 mg. of Pyribenzamine orally. A reduction in the reactivity of skin sites passively sensitized with serum containing cottonseed reagins was also observed.

Baer and Sulzberger (228) found that 150 mg. of Pyribenzamine orally reduced the dermographic response of a sensitive patient to a standard stimulus. Claman (229) showed that 150 mg. of Pyribenzamine definitely reduced the histamine flare reaction due to ragweed or histamine.

Feinberg and Freidlaender (188) noted definite improvement in 14 of 16 patients with dermographism after the administration of 50–100 mg. of Pyribenzamine.

Kurtin, Bierman, and Yontef (231) have shown that the iontophoresis of 5% Pyribenzamine prevents the erythema, edema, and vesiculation of normal patients exposed to ultraviolet light. They suggest that the drug be used in treatment of sunburn.

Feinberg and Bernstein (194) found Thenylene of benefit in 7 of 9 cases of dermographism, one case of pruritis of unknown origin, and 2 of 3 cases of pruritis ani. The experimental wheal and flare reaction of histamine or a specific antigen was decreased by local application of the drug.

Parrot (232, 233, 234) has shown that Antergan, 200–400 mg. per dose, inhibits the skin reaction due to burning, friction, viper venom, electrical stimulation, or injected histamine. Warembourge, Linquette, and Machon (235) have confirmed these results.

Thephorin, 50 mg. subcutaneously, prevented the itching but not the wheal formation caused by local application of one drop of 1:1000 histamine. Similar results were obtained with antigens after oral administration of the drug. In testing patients giving a positive dermographic reaction the same results were obtained (255).

Dysmenorrhea.—Eyermann (199) reported that dysmenorrhea was not ameliorated by Benadryl 50 mg. three times daily.

Serum Sickness.—Logan (202) found that 30 mg. but not 20 mg. of Benadryl were effective in treatment of a serum reaction in a 3-year-old child. Peterson and Bishop (230) reported that Benadryl, 50–100 mg. every six to eight hours, was effective in the treatment of serum sickness due to impure and pure horse serum and rabbit serum preparations.

Shock reactions due to injected allergens or liver extract were prevented by 100 mg. of Pyribenzamine prior to the injection. However, the drug was only a palliative (187).

Thephorin gave rapid relief to two patients suffering from serum sickness due to tetanus antitoxin injections (255).

Antistine has been used unsuccessfully in treating one case of "serum sickness" type reaction due to penicillin (256).

Migraine.—Benadryl, 50 mg. three times daily, had no effect on migraine (199). Barnett, *et al.* (201), obtained uncertain effects with Benadryl, 50–150 mg. daily, in two patients with migraine. Williams (200) found Benadryl of little value in myalgia

of the head. Migraine in adults was relieved with Benadryl 50–100 mg. every four to six hours. Similar results were obtained in children with a dose of 5–20 mg. two to four times daily. Although Levin (179) was successful in 9 of 15 adult cases and 2 of 3 children, he found that Benadryl aggravated the condition in two instances. Waldbott (204) used Benadryl to relieve 2 of 4 cases of migraine.

Arbesman, *et al.* (187), found Pyribenzamine of little value in migraine or histamine cephalalgia. This has been confirmed (253).

Neoantergan is of little value in the treatment of migraine (176).

Allergic headache is generally not benefited by Antistine (256).

Meniere's Disease.—Williams (200) obtained no results in the treatment of two cases of Meniere's disease with Benadryl.

Pyribenzamine was not beneficial in Meniere's disease (187). Epstein (190) also found that Pyribenzamine and Benadryl were ineffective in treating Meniere's disease.

Allergic Conjunctivitis.—Barnett, *et al.* (201), obtained improvement in 7 cases with Benadryl, 50–150 mg. daily. Benadryl gave complete relief to 2 patients and partial relief to one suffering from this condition (204).

Friedlaender and Friedlaender (256) showed that local application of a 0.25–0.5% solution of Antistine gave symptomatic relief in allergic conjunctivitis. The work confirmed the earlier observations of Bourquin (257).

Toxicity.—In general, toxicity due to overdosage or idiosyncrasy to antihistamine drugs is the same The symptoms commonly observed are dizziness, jitteriness, somnolence, dry nose, nausea, weakness, palpitation, insomnia, abdominal cramping, numbness, cold extremities, fatigue, tendency to hemorrhage, chloroform taste, faintness, acute hysterical reaction, dilated pupil, stupor, narcolepsy, sore tongue, hot flushes, early menses, dermatitis medicamentosa (175–177, 183, 184, 187, 192, 194–196, 206, 236–240, 253–256).

Brown (258) has shown, in a controlled study, that there is a definite psychic factor to be considered when evaluating the side effects caused by antihistamines. He found that placebos caused almost as many responses as Pyribenzamine.

Geiger, Rosenfield, and Hartman (241) reported upon the use of Benadryl in the treatment of pruritis associated with a generalized seborrheic dermatitis of unknown origin. The patient complained of palpitation, dimmed vision, malaise without drowsiness, and heartburn with nausea after receiving a total of 300 mg. of the drug in three days. After the administration of an additional 50 mg. of the drug the patient was found unconscious in bed, cold, pale, pulseless, and blood pressure could not be obtained, eyes were staring but pupils were equal and reacted to light. The administration of 0.46 cc. of a 1:1000 epinephrine solution subcutaneously caused the pulse to become palpable within thirty minutes and the patient became normal in three hours. Four days later the patient again was given Benadryl (50 mg. three times daily) and when 300 mg. had been administered the milder symptoms recurred.

Weil (242) observed acute toxicity due to Benadryl in a 3-year-old boy who had received 150 mg. of the drug in a period of six hours. Twenty minutes after receiving the last 100 mg. the child was found stitting up in bed, singing, laughing, starry-eyed, and in an apparent drunken condition. This was followed by muscular twitchings of the face, involuntary spastic movements of the extremities, and urinary incontinence. The muscular movements became worse, the child became irrational and dove out of bed on his head, his speech became slurred and hypertonic patellar and triceps reflexes were abnormal (all others were normal). The administration of 90 mg. of Seconal orally produced sleep but the muscular hyperactivity was such that the child had to be restrained. He became normal the following afternoon, sixteen hours after taking the last dose of Benadryl.

Schwartzberg and Willerson (243) observed toxicity in a man 38 years old who was taking 50–150 mg. of Benadryl daily. The usual toxic symptoms in varying degrees occurred along with an increased amount of flatus and three stools daily. Physical examination revealed a blood pressure of 100/64, edema of the eyelids, tenderness over the median nerve in both forearms and questionable edema of the forearms and hands. Reflexes were normal and except for the above the patient appeared normal. Even after the drug had been discontinued it was three months before the patient was completely recovered from the effects of this self-medication.

Borman (244) reported on an 18-year-old woman who took about 2000 mg. of Benadryl in forty-eight hours. Lethargy, mental confusion, and disorientation were observed but other than those symptoms of toxicity the woman was normal. Complete recovery occurred in forty-eight hours after stopping the drug and giving fluids freely, combined with several cups of strong coffee.

Swartz (245) treated a patient 32 years old for hayfever with Benadryl and observed the usual side reactions due to the drug. However, after a total dosage of 550 mg. of the drug the following symptoms occurred: nausea, vomiting, sense of rotation upon opening his eyes, marked rapid nystagmus, bilateral tinnitus, cold clammy skin wet with perspiration, blood pressure 90/60, and vertigo. Benadryl was stopped and $^3/_8$ gr. of ephedrine administered orally every two hours. Patient had same symptoms the following day when 9 Gm. of ammonium chloride daily was substituted for the ephedrine. He was placed on a high-protein, high-potassium, salt-free, and fluid-restricted diet. Upon the third day the symptoms were slightly abated but the blood pressure remained 90/60. The patient was not entirely free of the labyrinthine symptoms until the tenth day after stopping the Benadryl. Seven days after recovery the patient was given 20 mg. of Benadryl on an empty stomach and within thirty minutes the symptoms of toxicity returned. Treatment consisted of 0.3 cc. of epinephrine every half-hour for two hours. He was not symptom-free until four hours later. Four weeks later the patient took 25 mg. of Benadryl with the same results within thirty to forty minutes. This time the symptoms remained for eight hours but were controlled with epinephrine.

A case of a 3-year-old girl who swallowed 700–800 mg. (50–60/Kg.) of Benadryl was reported by Duerfeldt (246). The usual symptoms of acute toxicity

were observed and in addition there were convulsions and respiratory paralysis (4 times in less than four hours). Artificial respiration maintained the respiration and rectal administration of ether controlled the convulsions. The ataxia required four days to disappear but repeated examinations revealed no brain damage.

Sternberg (247) reported that a student nurse being treated for hayfever with Benadryl (50 mg. four times daily) experienced depression and confusion. On her day off she took three extra doses becoming drowsy, weak and amnesic, then hysterical a few hours later. For three days she had the same symptoms, crying spells, rubbed her eyes violently, and slept in spells. All this time the original dosage of the drug was continued. When the drug was stopped recovery occurred within forty-eight hours.

Eczematoid dermatitis from the ingestion of Pyribenzamine has been reported by Harris and Shure (248). After taking 50 mg. three times daily for six days the woman experienced a vesicular dermatitis on the dorsal surfaces of both hands, anterior surfaces of both thighs, and on the anterior surface of the chest. Also there was an intense pruritis. This dermatitis could be produced at will with the administration of 50 mg. of the drug, but skin tests were all negative. Epstein (249) observed a similar reaction in two patients who were receiving 50 mg. of Pyribenzamine three times daily. One developed a dermatitis resembling pityriasis rosea and the other a widespread erythematopapular eruption.

The development of fever and granulocytopenia in an elderly patient who was taking 200 mg. of Pyribenzamine daily was reported by Blanton and Owens (250). Scratch tests were negative but *in vitro* tests indicated that the drug was causing destruction of the leucocytes. The total leucocyte count and differential percentage returned to normal twelve days after the drug was stopped.

Kern (259) reported a case of toxicity due to Pyribenzamine in which the patient fainted after taking one dose of the drug. She continued the drug for a total of three doses, having the same difficulty after each dose. Although she remained prostrate all that day, recovery occurred the morning following withdrawal of the drug.

Peirce and Mothersill (196) gave 5 patients an average daily dose of 170 mg. of Thenylene for a period of three months. They found no evidence of damage to the blood, heart, liver, or kidneys.

Ghiselin (215) reported that Anthallan in total average dose of 11.65 Gm. over a period of seven of thirty-five days had no effect on either the blood chemistry or hematological constituents of the patients treated.

SUMMARY

A review of chemistry, pharmacology, and clinical usage of the antihistaminic drugs has been presented. It is difficult to compare the antihistaminic properties of the drugs because the methods employed by the various investigators were not always the same. From a general pharmacological viewpoint more investigation should be undertaken to determine the site of action of the drugs alone upon the various body systems. Clinically the antihistaminic drugs appear to be beneficial in the treatment of hayfever, vasomotor rhinitis, drug rashes, pruritis, contact dermatitis, acute and chronic urticarias, atopic dermatitis, and allergic conditions causing a histamine flare reaction. However, the results obtained in the treatment of asthma and in histamine-induced gastric secretion are disappointing and indicate that the drugs are either not true antihistaminics, are unable to reach the site of action of histamine on the particular body systems involved, or that some other agent causes the symptoms attributed to histamine. The toxic effects caused by all these drugs are similar and the physician as well as the patient should bear in mind that excessive dosage is dangerous.

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The Quantitative Determination of Crystalline Penicillin G by Infrared Analysis*

By EDWARD A. GARLOCK, JR., and DONALD C. GROVE†

The quantitative analysis of commercial samples of crystalline sodium, potassium, and buffered penicillins for the penicillin G content by means of the infrared spectrometer is presented. The analysis is conducted on a suspension (mull) of the crystals in liquid petrolatum. The proportion of the liquid petrolatum and the crystalline penicillin is kept constant and a cell of known thickness is used, thus eliminating the necessity of an internal standard. The same mull can also be used to identify other types of penicillin present in the sample. The spectra for potassium penicillin G, dihydro F, F, K, and X are given. Results obtained using the infrared technique and the N-ethyl piperidine method for the determination of penicillin G are compared.

THE COMMERCIAL production of infrared spectrometers has made easily available to the chemist a new tool with which not only the qualitative characterization of organic molecules but also quantitative determination of them in mixtures can be obtained. The application of infrared analysis to the crystalline penicillins, both from a qualitative as well as quantitative aspect, has been reported by Barnes, *et al.* (1). In their procedure, the sample of penicillin is analyzed in the solid phase after mulling with liquid petrolatum between rock-salt plates. Quantitative analysis of sodium penicillin G is made, using *dl*-alanine as an internal standard. The purpose of the internal standard is to eliminate the necessity of having to know the length of the absorption cell. The analytical results for known mixtures of sodium penicillin G and magnesium oxide are reported, but no results are given for the determination of sodium penicillin G in the presence of known amounts of the other penicillins. Errors due to crystal orientation and difficulties of thoroughly mixing the sample and standard are discussed.

EXPERIMENTAL

It has been our experience in following the technique of Barnes, *et al.* (1) that variations in the thickness of the mull between the rock-salt plates are not fully compensated for by the internal standard. If the film is a little too thick or too thin, inconsistent results are obtained. In addition, it is difficult to obtain a mull of a fixed ratio between the two solid phases and the liquid petrolatum. Furthermore, examination of many commercial samples of crystalline sodium penicillin, crystalline sodium penicillin buffered with sodium citrate and crystalline potassium penicillin has shown variable absorption in the wave-length region chosen by these authors to measure the absorption of *dl*-alanine. One of the purposes of the present paper is to report a technique in which an absorption cell of known length is employed obviating the necessity of an internal standard.

Analysis of crystalline compounds in the solid state in the infrared is complicated by physical factors not encountered in the case of liquids, solutions, or gases. Reflection and strong scattering of the incident light, the type and size of the crystals as well as the nature of amorphous or crystalline impurities may play important roles. It thus becomes necessary to follow an exacting and standardized technique in order to obtain accurate and reproducible results.

Absorption Cell.—The absorption cell used consists of two circular rock-salt plates two inches in diameter, separated from each other by means of a brass spacer. The cell is held tightly between two metal plates (cell holder) by means of threaded studs and nuts. The metal cell holder is illustrated with dimensions in Fig. 1. A brass spacer was

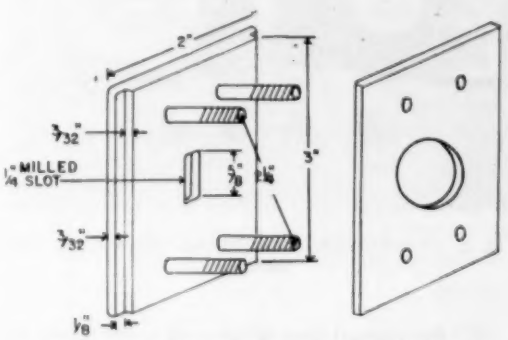

Fig. 1.—Details of absorption cell holder.

chosen in preference to the usual lead spacer because it is stiffer, thus more easily handled; in addition, it can be used repeatedly without change in thickness. The spacer thickness should be between two- and three-thousandths of an inch. The exact thickness need not be known if the same spacer is used for both the standard and the unknown sample. The spacer is cut in the shape of a circular gasket with a 1-inch center hole and a slit to permit the escape of air when the two cells are clamped together. In

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order to further standardize the procedure, the ratio of the penicillin to liquid petrolatum is kept constant. A satisfactory mull is obtained by using a ratio of one part of penicillin to two to three parts of liquid petrolatum. Since the concentration and cell thickness are fixed, it is possible to measure the penicillin G content of crystalline penicillin from the infrared absorption at only one wave length. If desired, the complete infrared spectrum can be run and the amounts of other types of penicillin present can be estimated at the wave length characteristic for the type.

Procedure.—The data presented here were obtained, using a Perkin Elmer model 12-B infrared spectrometer. The details of the procedure used are as follows:

1. Grind the sample to be analyzed with a mortar and pestle.
2. Weigh by difference 100–150 mg. of liquid petrolatum into an agate mortar. Multiply the actual weight of the liquid petrolatum by 0.4 and add exactly this amount of penicillin to the liquid petrolatum in the mortar.
3. Mix the sample and liquid petrolatum with a small spatula and then mull thoroughly with the pestle until a uniform consistency is obtained.
4. Lay the cell holder down with the studs up and place a 2-inch diameter salt plate between the studs. Center this plate with respect to the opening cut in the holder.
5. Lay the spacer (0.0024-inch thickness) on the plate (see Fig. 2).

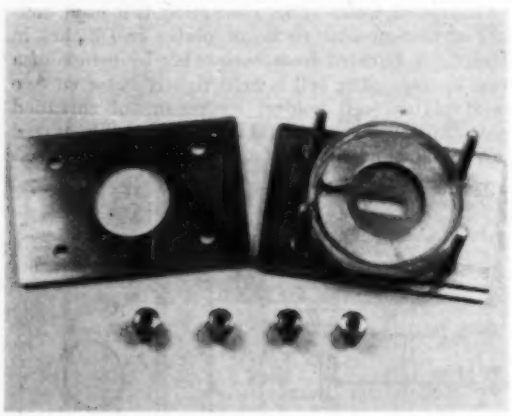

Fig. 2.—Photograph of absorption cell without the mull.

6. Put a small drop of the mull in the center of the plate, and bring the top salt plate down gently so as to spread the mull uniformly over the slit opening (see Fig. 3).
7. Place the metal cover over the assembled cell and screw the cover down tightly. (The spacer prevents change in thickness.)
8. Examine the mull by holding the assembled cell up to the light. It should appear smooth and free of any air bubbles and not in contact with the spacer. If it is not uniform or is in contact with the spacer, dismantle the cell, remove the mull, add a new drop and reassemble as indicated above.
9. Adjust the amplification of the spectrometer to full-scale deflection for one microvolt. Set the slit opening to 0.750 mm. and record the spectrum from 14.8 to 13.8 microns, being sure to take a zero reading (shutter closed) at the beginning and at the end of the run.

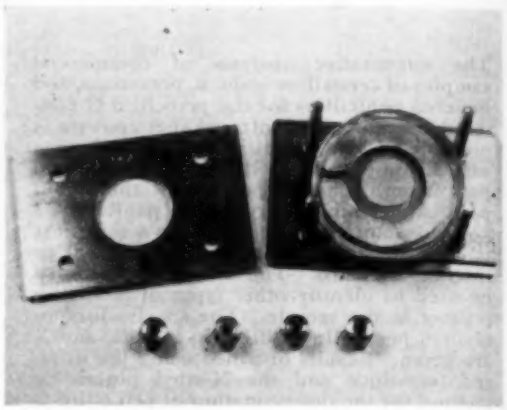

Fig. 3.—Photograph of absorption cell with the mull.

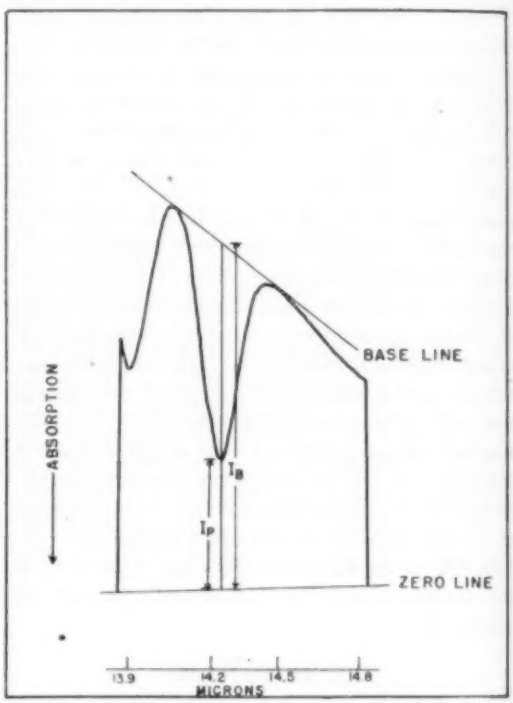

Fig. 4.—Method of calculating the base-line density for crystalline sodium penicillin G.

10. Draw in the base-line between two points, one on each side of the analytical band. As illustrated in Fig. 4, calculate the base-line optical density, Wright (2), Heigl, *et al.* (3), from the equation:

$$D_B = \log_{10} \frac{I_B}{I_P}$$

where

D_B = base-line optical density.
I_P = distance from the zero-line to the maximum absorption of the band.
I_B = distance from the zero-line to the base-line measured at the same wave length as I_P.

Various combinations of the F.D.A. working standard of crystalline sodium penicillin G with either sodium penicillin F, sodium penicillin K, or sodium penicillin dihydro F were prepared. The base-line density of these mixtures was determined by the above method and when plotted against the concentration of sodium penicillin G, a linear relationship was obtained. The curve obtained by mixing the F. D. A. standard sodium penicillin G with a composite mixture of sodium penicillin F, K, and dihydro F is shown in Fig. 5. The appearance of the absorption band at the various concentrations of penicillin G is shown in Fig. 6. Thus, the determination of penicillin G is valid in the presence of penicillins F, K, and dihydro F. Sodium penicillin X which absorbs strongly in the region of 14.2 microns would cause some interference; however, as crystalline penicillin now being commercially produced does not appear to contain penicillin X, this presents no immediate problem. Penicillin X also shows absorption at 12.03 microns (Barnes, *et al.* (1)), and by measuring at this wave length a correction for the amount of penicillin X present could be made. The accuracy of the above method as determined by the analysis of known mixtures of sodium penicillin G and non-G is within 2.5%.

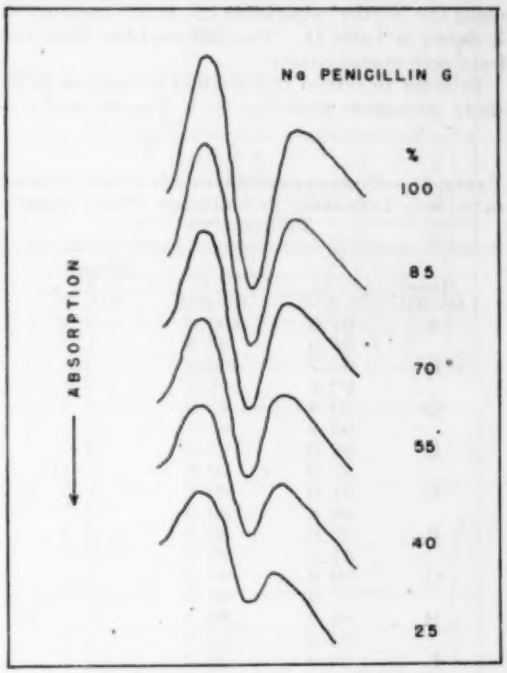

Fig. 6. Infrared absorption at 14.2 microns of crystalline penicillin G when mixed with non-G penicillin.

Base-line density values obtained for eight analyses of the standard sodium penicillin G during a period of two months, using the same cell and spacer, are as follows: 0.414, 0.398, 0.393, 0.402, 0.400, 0.404, 0.398, and 0.395. Thus for accurate routine work it is necessary to determine the base-line density of a standard sodium penicillin G from time to time to take care of changes in the spectrometer and the salt plates of the cell.

Table I shows the precision of this procedure. These results were obtained by analyzing each of two commercial samples of sodium penicillin G four times.

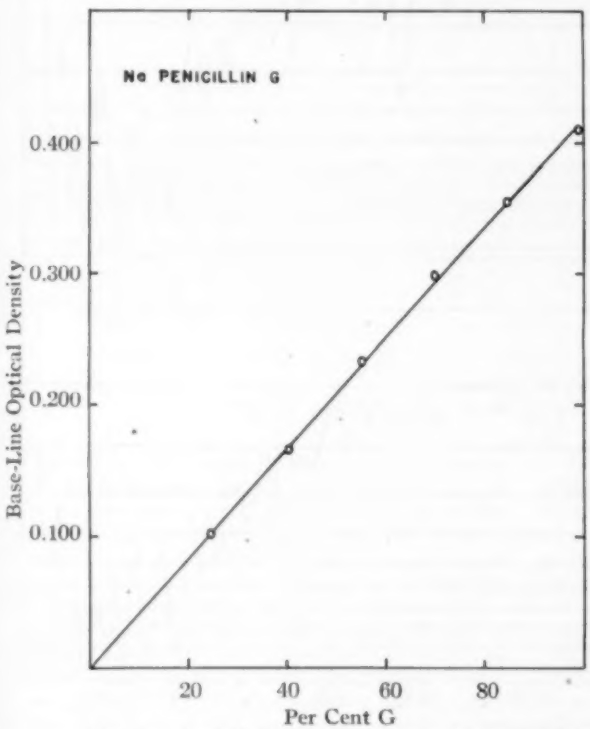

Fig. 5.—Analytical working curve for crystalline sodium penicillin G.

TABLE I—INFRARED ANALYSIS OF TWO COMMERCIAL SAMPLES OF PENICILLIN G

	Sample A, % G		Sample B, % G
	101.1		91.5
	97.2		95.0
	97.0		94.7
	101.7		93.5
Av.	99.3	Av.	93.6

The deviation of these values from the average is within 2.5% for both samples.

A comparison of values obtained for various commercial samples of crystalline sodium penicillin G by the above procedure compared with those obtained

using the N-ethyl piperidine (N. E. P.) method (4) is shown in Table II. Two different lots were run from each manufacturer.

In order to extend this method of analysis to include potassium penicillin G, it was necessary to obtain the various potassium penicillin salts of the other types of penicillin. We have converted the sodium salts of penicillins K, F, dihydro F, and X to the corresponding potassium salts by way of the free acids followed by the formation and crystallization of the potassium salts. All of these salts were crystalline and possessed the calculated biological activity, indicating a high degree of purity. However, because of the scarcity of the sodium salts of these penicillins, we were not able to characterize these potassium salts by more complete analytical data or establish their chemical purity. The units per mg., as determined by bioassay, were 2020 for the potassium penicillin K, 1684 for the dihydro F, 743 for the X, and 1341 for the F.

TABLE II.—COMPARISON OF THE N-ETHYL PIPERIDINE AND INFRARED PROCEDURES USING SODIUM PENICILLINS

Manufacturer	% G, N. E. P.	% G, Infrared	Difference, I. R. − N. E. P.
A	97.0	100.0	+3.0
	95.0	93.8	−1.2
*B	95.7	92.5	−3.2
	99.0	101.0	+2.0
C	90.8	85.0	−5.8
	90.8	83.7	−7.1
D	92.0	83.0	−9.0
	81.0	79.6	−1.4
E	91.0	89.5	−1.5
	96.8	99.3	+2.5
F	92.6	96.5	+3.9
	91.5	93.6	+2.1
G	99.6	97.5	−2.1
	97.8	98.5	+0.7
H	88.0	85.5	−2.5
	92.5	92.0	−0.5
I	95.0	96.0	+1.0
	90.0	85.5	−4.5
J	96.0	103.0	+7.0

The potassium penicillin G used in this work was supplied by one of the manufacturers who reported the following analytical data:

Potency

Biological Assay	1587 u./mg.
Iodometric Assay	1591 u./mg.

"G" Analysis

Craig extraction	100.0%
N. E. P. weight	100.4%
Ultraviolet	100.0%

The infrared spectra of these potassium penicillins are shown in Figs. 7 and 7*a*. These are the actual recordings of the spectra, using a motor-driven slit control mechanism which gives a nearly level energy

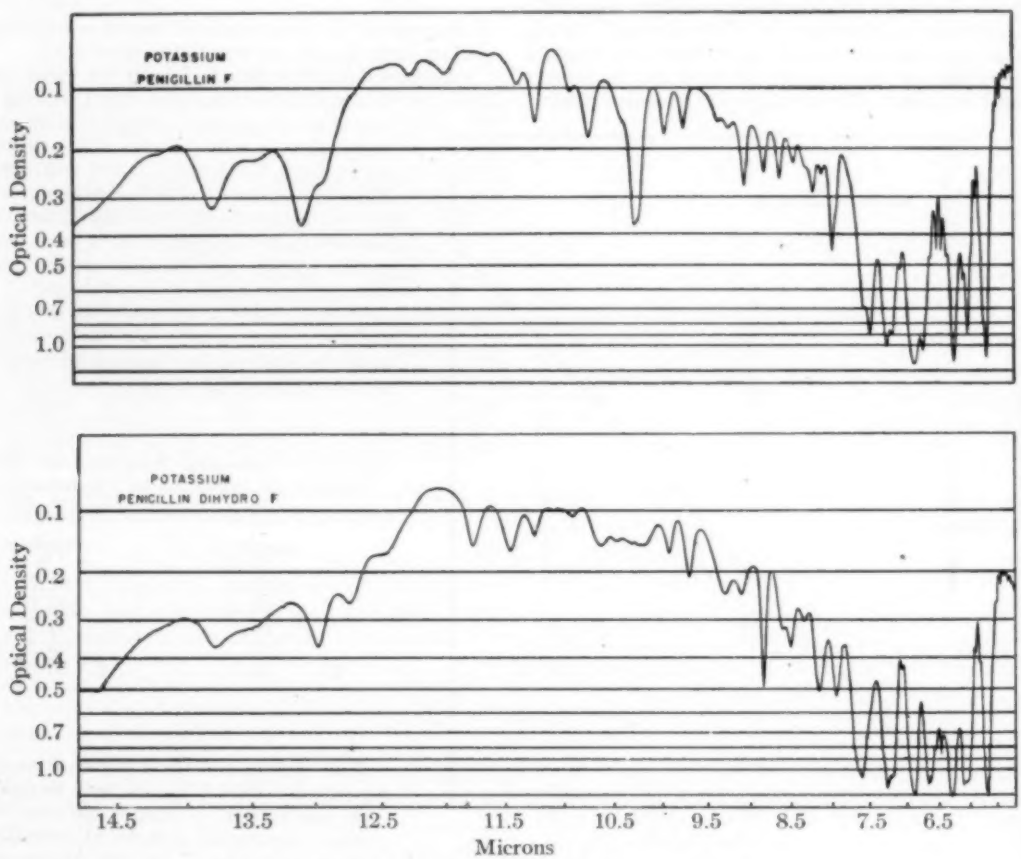

Fig. 7.—Infrared spectra of crystalline potassium penicillins.

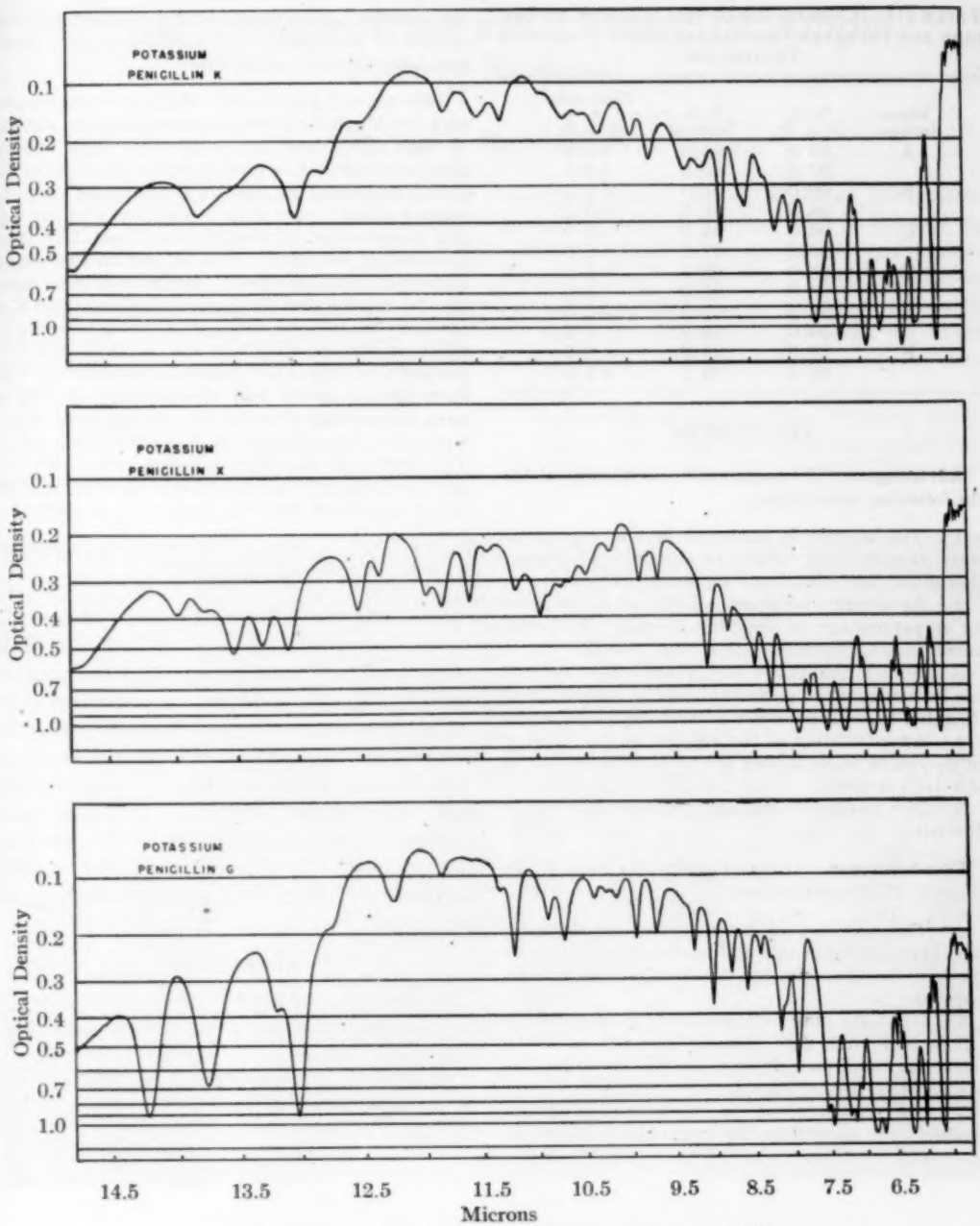

Fig. 7*a*.—Infrared spectra of crystalline potassium penicillins.

background. The approximate optical densities are indicated on the recordings.

Using the procedure described above for sodium penicillin G, a working curve was prepared for potassium penicillin G diluted with potassium dihydro F. The results show a linear relation between the base-line optical density and percentage of penicillin G. A comparison of the values obtained by analyzing a series of commercial samples of crystalline potassium penicillin G by the infrared procedure and by the N-ethyl piperidine method is given in Table III. The results are slightly higher by the infrared method.

Commercial samples of crystalline sodium penicillin G buffered with sodium citrate prepared by three manufacturers were analyzed for penicillin G content by this infrared procedure. Good results were obtained on the samples of two of the manufacturers while the samples of the third manufacturer gave inconsistent results. These latter samples appeared to have been ground to a very fine powder which would not give a smooth liquid petrolatum mull and consequently gave poor analytical results. However, after recrystallization from 95% methyl ethyl ketone, satisfactory analyses were easily obtained.

TABLE III.—COMPARISON OF THE N-ETHYL PIPERIDINE AND INFRARED PROCEDURES USING POTASSIUM PENICILLINS

Manufacturer	% G, N. E. P.	% G, Infrared	Difference, I. R. − N. E. P.
A	97.0	99.0	+2.0
	97.0	99.0	+2.0
B	96.0	97.0	+1.0
	98.0	98.5	+0.5
C	96.0	94.5	−1.5
	95.5	97.5	+2.0
D	86.0	92.5	+6.5
	90.0	91.0	+1.0
E	94.5	97.0	+2.5
	99.0	98.0	−1.0
F	89.5	96.0	+6.5
	91.5	96.5	+5.0

DISCUSSION

Advantages.—The proposed infrared method has the following advantages:

1. The sample is analyzed in the crystalline state, thus it is not subject to any possible changes that might take place in extraction procedures.
2. An accuracy of about ±2.5% can be obtained for all percentages of penicillin G when mixed with known non-G penicillins other than penicillin X.
3. Both a qualitative and quantitative analysis can be made on the same mull, thus a knowledge of the other types of penicillin present can be obtained.
4. An estimation of the amount of non-G penicillins can be made if they are present to the extent of 5–10% or more.
5. The method is fast and the technique is not difficult.

Disadvantages.—It may be said that some of the possible disadvantages are:

1. The necessity of having pure standards for each type penicillin salt and having to redetermine the base-line densities for the standards at various intervals.
2. Difficulty may be experienced in preparing a satisfactory mull, if the sample has been milled to extremely small particle size. In this case, one has to resort to recrystallization.
3. Uncertainty as to the effects of degradation products and accompanying impurities in commercial samples. This might include compounds such as salts of penicilloic acid, penillic acid, etc., which were not available for this study.

This method gives results which are in agreement with the N-ethyl piperidine procedure when samples of high G content are compared. The N-ethyl piperidine method requires careful attention to details (i.e., careful selection of solvents and reagents, control of the temperature of solutions and the time and temperature of precipitation) and, like other gravimetric procedures, it is subject to solubilizing and coprecipitation effects. It has been observed in this laboratory that the presence of penicillin K in amounts of 10% or more produces a solubilizing effect on the precipitate in the N-ethyl piperidine method resulting in low values for penicillin G. We have also observed that the presence of 10% or more of penicillin F or dihydro F may cause coprecipitation, thus giving rise to high penicillin G values. The amount of the coprecipitation varies with temperature, different batches of solvent used, and different lengths of time the precipitate is allowed to stand.

SUMMARY

The quantitative infrared analysis of penicillin G in commercial samples of crystalline sodium, potassium, and buffered penicillins has been described. A mull of definite concentration is analyzed in a cell of constant thickness, thus eliminating the necessity of an internal standard. The prepared mull is suitable for both qualitative and quantitative analysis. The absorption spectra of the potassium salts of the penicillins are recorded. A comparison of this infrared method with the N-ethyl piperidine method has been made.

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(4) (a) Sheehan, J. C., Mader, W. J., Cram, D. J., *J Am. Chem. Soc.*, **68**, 2407(1946). (b) Mader, W. J., Buck R. R., *Anal. Chem.*, **20**, 284(1948). (c) *Federal Register*, Title 21, Chapter I, Part 141, Section 141.3 (f), (November 28, 1946).

WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

Epicarin
Mercaptopyruvic acid
Quinitol
Ribose-*l*-phosphate
Dambonitol
Sequovtol
Scyllitol
Conduritol
Inosinic acid
Xanthylic acid
Uridine
Cytosine desoxyriboside

Aseptic Operation and Control of Ampul Filling Rooms*

By JOHN H. BREWER

In our attempts to maintain sterility of our products, we, as manufacturers, have employed all sorts of devices to aid us in this effort. With the help of over-zealous salesmen, we have installed so-called "sterile" rooms, precipitrons, germicidal lamps, aerosol nebulizers, and other items, some of which closely approach witchcraft. We wrap ourselves in gowns resembling Roman togas; we take off our shoes in an outer chamber as if we were entering some oriental temple, and go through a hand-cleansing ceremony anointing ourselves with bacteriostatic agents. All of this is done to avoid some invisible devils often referred to as pyrogens or contaminations.

We do this all too often without having actual proof of its value. In order to be practical about many of these procedures, about ten years ago, after visits to most of the pharmaceutical laboratories, we constructed some dust-free rooms on a slightly different pattern from that used by most manufacturers of biologics and set up a system for determining the prevalence of bacteria in these cubicles.

Room Construction.—These rooms, shown in Fig. 1, are so constructed as to permit easy cleaning, there being no table legs or furniture with the exception of the work shelves and movable chairs. All joints are rounded coves and the glass is imbedded in mastic. The doors are felt- and copper-stripped and the air inlet is by means of a Carrier Model J Air-Conditioner with oil-soaked glass wool filter. The exhaust is protected by the same filter and entrance is made through air locks. The ceilings are sloped so that if aerosol sprays are used any condensation will run off and not drip down on operators' necks. The equipment in

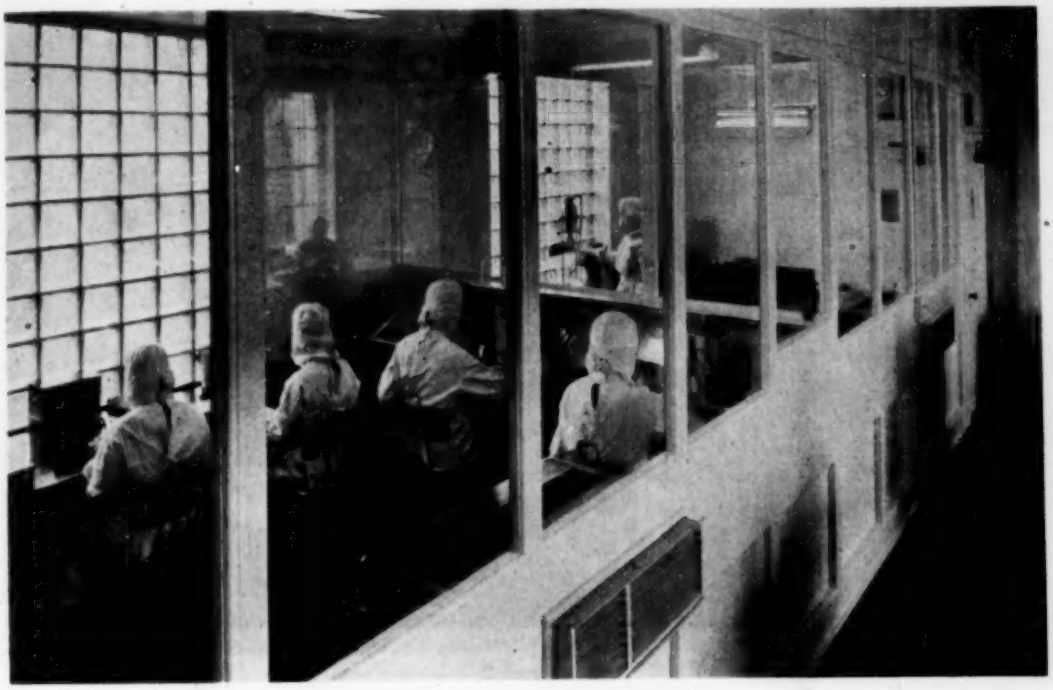

Figure 1.

* Presented to the joint meeting of the Scientific Section and the Biological Section of the American Drug Manufacturers Association, thirty-sixth annual meeting, Hotel Mount Washington, Bretton Woods, N. H., June 7, 1948.

Received June 24, 1948, from Biological Research Laboratories, Hynson, Westcott & Dunning, Inc., Baltimore, Md.

the room is so arranged that five operators are required; two at filling positions and three at sealing devices (Figs. 2 and 3).

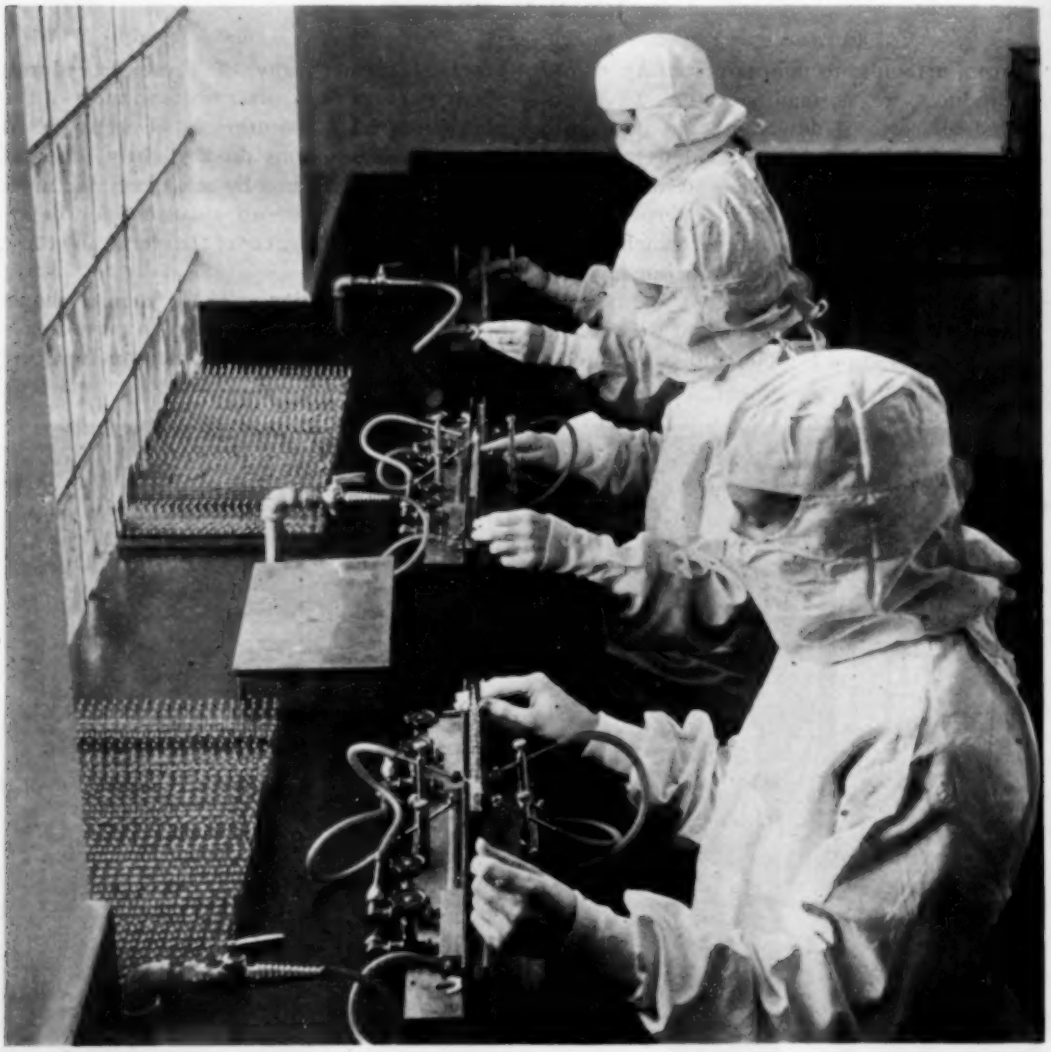

Figure 2.

Testing System.—We realize that occasional exposure of plates in these rooms would be of little value in determining the usefulness of the above equipment and techniques, so that a routine system of testing was instigated. We found at first that the use of plain agar plates was of little value, since most of the contamination came from the operators themselves. Blood agar plates were then tried and the counts increased enormously. Daily, four blood agar plates and two anaerobic plates (using anaerobic agar and an anaerobic lid) are exposed for fifteen minutes. These six plates are arranged as shown in Fig. 4. At the position marked *1* under the glass shield where two fillers work, one blood agar plate and one anaerobic agar plate are exposed. At position *2* where the sealing occurs, another blood agar plate is exposed. At position *3*, an anaerobic plate as well as a blood agar plate are exposed. At position *4*, on the storage table, the last blood agar plate is exposed (Fig. 5). The standard fifteen-minute exposure does not occur at a set time daily, nor do the workers know exactly when the plates will be exposed each day. In this way a more representative count is obtained, since the girls are forced to continue working normally in order that they can meet the daily filling schedule requirements.

Next, the plates are incubated at 37.5° for forty-eight hours, and are then counted and identified, although this does not extend to serological classification. The total colonies per plate is recorded on the chart beside the position numbers, as shown in Fig. 6, and the total of all four blood agar plates is put in the space provided at the bottom. Thus, daily totals can be posted graphically, keeping the ampul workers aware of any rise in the counts and encouraging them to improve in technique. If the counts rise appreciably, it is very easy to convince the maids and porters that a better job of cleaning must be done.

By again looking at Fig. 4 it can easily be seen how a record of where each worker is located during the exposure time would be of great aid in detecting any one of them who might be disseminating large numbers of bacteria without apparent physical ill effects, yet running up

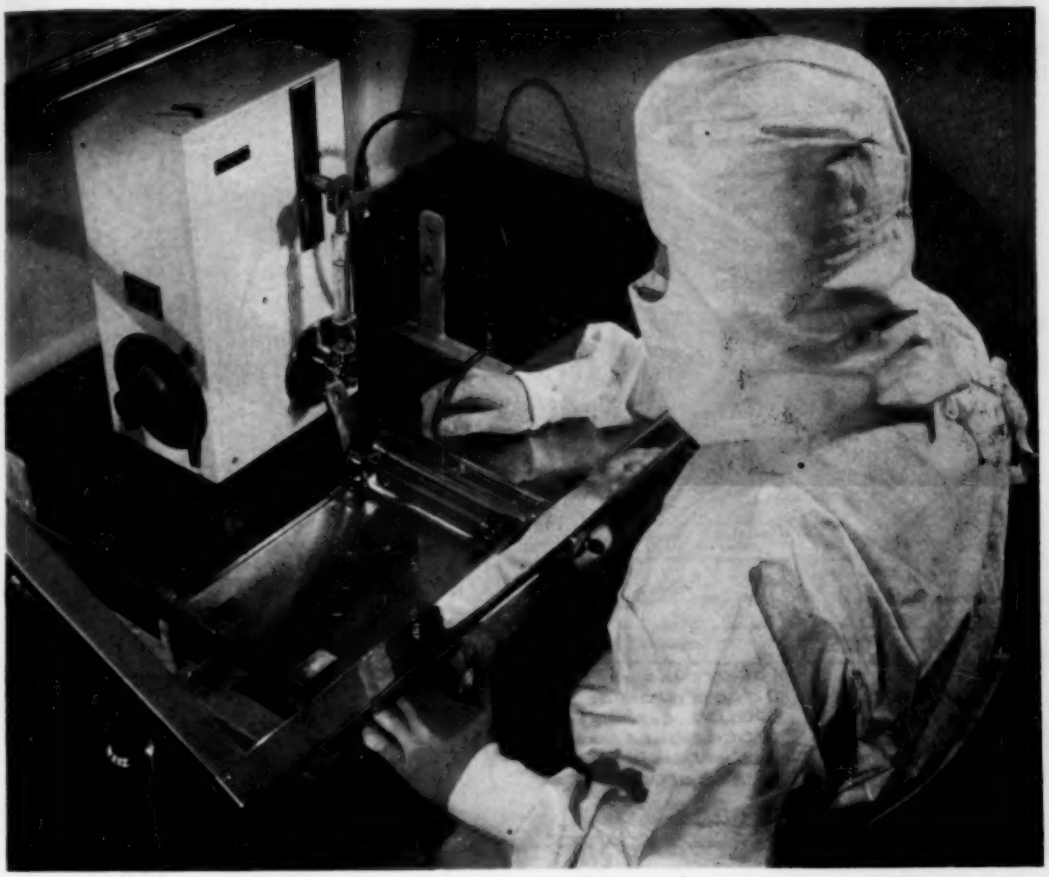

Figure 3.

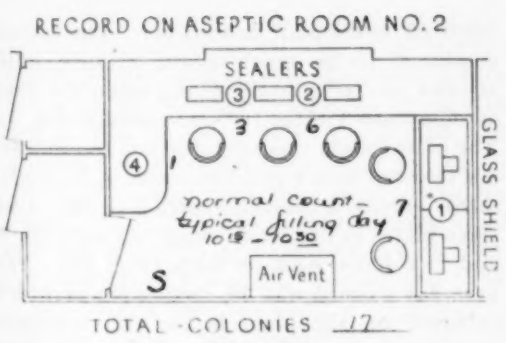

Figure 4.

the count and possibly contaminating the products. Concrete proof of the effectiveness of this method of check has been obtained several times and is illustrated by the following case.

The counts were noticed as being particularly high for about a two-week period, culminating in an extraordinary increase on the day of a sterile filling. The total count for the four aerobic blood agar plates was 164 colonies (Fig. 7). On the plate immediately adjacent to one of our operators the colonies num-

bered 90—over half the total. The other three plates showed only 23, 28, and 23 colonies. The counts proceeded normally until another alarmingly high count appeared on the day of an unsterile fill (Fig. 8). The total count was 172 for the four blood agar plates, 139 of which appeared by the worker in question. The next day's count was 145, sixty-four of which were on the plate exposed beside this operator. The count continued to be high for two more days but suddenly dropped to 9 colonies on a third day when the operator in question did not work in the filling rooms. On her return the count rose to 47, thirty-two of which

Figure 5.

Aseptic Room #2 Date 5/24/57

Sample

Plate No.	Exposure Period	Number of Colonies	Organisms
1	15 min	4	2 staph albus 2 diptheroids
2	"	3	1 staph citreus 2 staph albus
3	"	5	3 diptheroids 2 staph albus
4	"	2	2 staph albus

Notes on Reverse Side Tested by G. E. Longest

Figure 6.

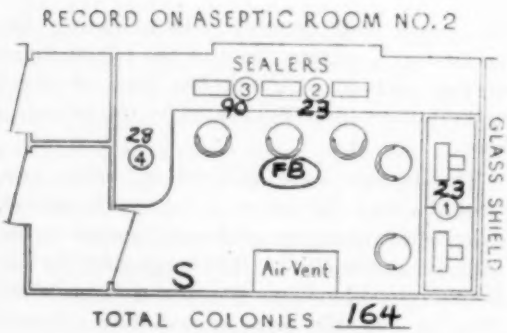

Figure 7.

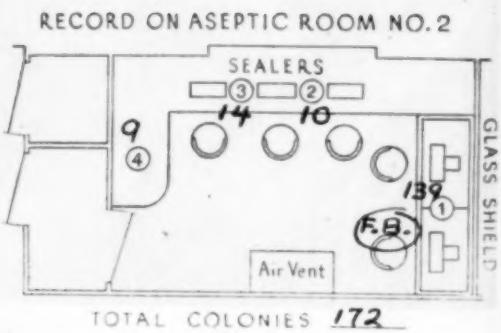

Figure 8.

occurred on the plate nearest her. The counts continued to be much above average until another day of a sterile filling when the four aerobic plates yielded 160-plus colonies (Fig. 9). The plate beside this operator was too numerous to count with a preponderance of *Staphylococcus aureus*, a decided contrast to the other three plates in the room which had only 28, 8, and 11 colonies each on them (Fig. 10). The next day's count was 88, forty-eight of which occurred on the plate beside this operator. She was removed from the filling staff, after

which the counts again dropped to normal. Throughout this time, control plates were poured under exact conditions and these remained sterile. To insure further against error, each worker was taken separately into another dust-free room where she worked for fifteen minutes, surrounded by four blood agar plates. The average total four-plate count for the worker in question was 59. Figure 11 shows the highest count obtained on her. The average for the other girls was 16. Figure 12 shows a typical count. This operator was put on penicillin therapy until such time as she could undergo a tonsillectomy. The surgeon who removed her tonsils reported them as

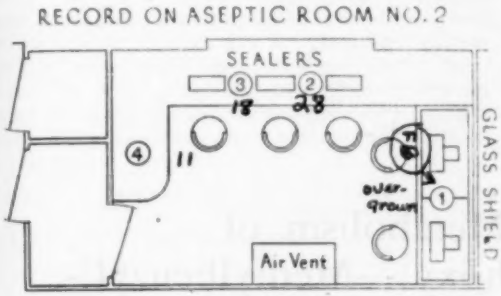

Figure 9.

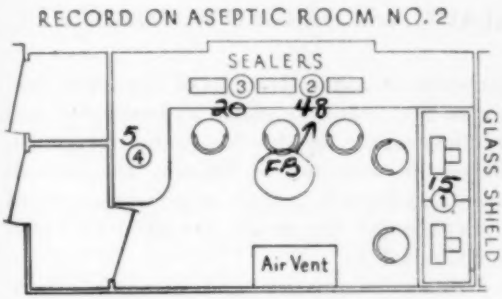

Figure 10.

being highly infected. Following this operation, her count returned to normal.

Two-year Record.—The following statistics have been compiled on the basis of the last two years' records of this daily six-plate exposure method. The average total count of the four aerobic blood agar plates for a sterile fill is 20 colonies, or 5 colonies per plate per fifteen minutes. The average total count on an unsterile fill is 27 colonies, or approximately 7 colonies per plate per fifteen minutes. (The only difference between a "sterile" and "nonsterile" fill is that no mask is worn by the workers during an "nonsterile fill" and all products ampuled by a "nonsterile fill" are autoclaved afterward. On a "sterile" fill, the workers are masked completely except for the eyes, and precautions about keeping down bacteria are more rigid.)

Considering the types of bacteria which appear on these plates, we have found that *Staphylococcus albus* (hemolytic and nonhemolytic varieties), diphtheroids, *Staphylococcus aureus*, and *Staphylococcus citreus* appear in that order of frequency. Very few Gram-positive spore-forming bacilli have been picked up. During the entire year 1946–1947 no spore formers were picked up on the plates. Molds and fungi of various types, streptococci, and

Aseptic Room F.B. - Typical Count Date —

Plate No.	Exposure Period	Number of Colonies	Organisms
1	15 min.	24	8 Hemolytic Staph 10 Staph albus 6 diphtheroids
2	"	25	8 Hemolytic Staph 11 Staph albus 6 diphtheroids
3	"	35	10 Hemolytic Staph 8 Staph aureus (citreus) 4 Staph albus 7 diphtheroid 1 mixed gray & red 13 Staph
4	"	30	8 Hemolytic Staph albus 4 Staph aureus 4 diph

Notes on Reverse Side (14) - total Tested by A. Clough

Figure 11.

Aseptic Room Girls - Average Count Date —

Plate No.	Exposure Period	Number of Colonies	Organisms
1	15 min.	3	1 Staph albus 2 diphtheroids
2	"	5	3 Hemolytic Staph 2 Staph albus
3	"	3	1 Staph aureus 2 Staph albus
4	"	2	1 Staph aureus 1 diphtheroid

Notes on Reverse Side (13) - total Tested by A. Clough

Figure 12.

sarcinae are found from time to time. No anaerobic organisms have been identified other than facultative organisms of the same types found on the aerobic plates.

This technique has become invaluable to us in that the average count in various seasons with different products has been in existence for so many years that it affords us an opportunity to test various new aseptic equipment and prove or disprove such things as cold cathode as opposed to hot cathode "sterilamps"; the use of glycol waxes or mopping aids, different types of aerosol spraying equipment; the use of gowns with stockinet sleeves as opposed to loose sleeves, and the value of various types of masking equipment.

SUMMARY

We have found that although the name "steri-lamps" is a misnomer, if left burning continuously, they will reduce the normal count 50% in our particular setup. This is true of both the cold and hot cathode type, although we have a slight preference for the hot cathode type since fixtures available for these seem safer and more satisfactory.

As to the gowns, we find a stockinet sleeve, wrap-around type operating gown which ties in the rear with a Hopkins-type operating hood and mask combined gives us consistently lower counts than other types.

We do not feel that at the present state the use of aerosol nebulizers is warranted.

We have found the best general check on our rooms, equipment, and technique is periodically to autoclave 8 liters of Trypticase soy broth (the best culture medium we know how to make), give it a stock number, and give it to the girls to fill into ampuls. When they have finished, place all the ampuls in the incubator and see how many show growth after seven days' incubation. This is a challenging project and, if done periodically, should build confidence or cause the manufacturer to take corrective measures immediately.

Studies on the Metabolism of 2-[α-(2-Dimethylaminoethoxy)-α-Methylbenzyl]-Pyridine Succinate (Decapryn Succinate)[1, *]

By FRED H. SNYDER, GERTRUDE R. KLAHM, and HAROLD W. WERNER

Experiments in rats and dogs, employing various dosage regimes, demonstrate urinary elimination accounts for roughly only 10 to 30 per cent of administered Decapryn Succinate. The antihistamine does not accumulate in various body tissues, and it is concluded that the bulk of administered Decapryn Succinate is destroyed in the body.

PHARMACOLOGICAL and clinical studies on a new antihistamine compound, 2-[α-(2-dimethylaminoethoxy)-α-methylbenzyl]-pyridine succinate (Decapryn Succinate), have recently been reported (1–7). The present paper deals with investigations of the fate of this compound following administration to laboratory animals.

METHODS

Animal Work.—Studies have been carried out on urinary elimination and tissue concentrations in white rats and mongrel dogs. Evaluations in rats were made following single intravenous administrations of Decapryn Succinate and following single and repeated oral administrations of this histamine antagonist. Urine was collected by housing the animals over large funnels, and the urine was separated from feces by the device described by Gross and Connell (8).

Urinary elimination and in some experiments blood levels were determined for dogs which had received Decapryn Succinate in hard gelatin capsules three times a day for thirty to fifty days. Animals were housed in the usual type of metabolism cage for urine collection.

Analytical Procedure.—The picric acid colorimetric method of Richter (9) was used for the determination of Decapryn in urine and tissues. This is a general method for aliphatic amines, and color production is not necessarily limited to unchanged Decapryn. Degradation products may thus be responsible for a portion of the color produced, but the results give important information regarding the appearance of extra amine in the urine.

Recovery experiments have demonstrated

[1] This trade name of The Wm. S. Merrell Co. will be used throughout the paper for convenience.

[*] Received June 22, 1948, from the Pharmacology Department, Research Laboratories, The Wm. S. Merrell Co., Cincinnati, Ohio.

that the method is accurate for the determination of Decapryn Succinate to within 3 per cent. Other studies have shown that the excreted product is stable in urine at room temperature for at least the twenty-four-hour period employed in collecting specimens.

EXPERIMENTAL RESULTS

Excretion Studies in Control Rats.—Twenty-four-hour urine samples were collected individually from 25 normal adult rats of varying age and weight and of both sexes, and samples were assayed by the colorimetric procedure. The amount of chromogenic material excreted, expressed as Decapryn Succinate, varied between 0.1 mg. and 0.2 mg./day, with an average of 0.15 mg./day. This quantity is not significant in those experiments where moderate amounts of amine are excreted, but it assumes some importance when small doses are administered or when delayed excretion is measured. Consequently, in order to avoid attaching undue significance to trace excretion, an arbitrary control value of 0.2 mg. was subtracted from each twenty-four-hour figure obtained with experimental animals.

Excretion Following Intravenous Administration to Rats.—The excretion of Decapryn following its introduction directly into the blood stream was determined in a group of 12 animals. The dose employed, 25 mg./Kg., produced some signs of toxicity, but no deaths resulted. The use of smaller doses was not feasible because the amounts excreted would have fallen too close to the control level.

As shown in the upper part of Table I, the 6 male rats excreted an average of about 8%, and the 6 female rats excreted an average of about 17% of the administered dose in twenty-four hours. This difference appears to be statistically significant.

Excretion during the second day following drug administration was very low, averaging less than 1% of the dose, and there was no significant difference between the sexes.

Excretion Following Single Oral Doses to Rats.—Three dose levels of Decapryn Succinate and a total of 56 animals were used in the studies.

Table I shows that the percentage elimination was lowest with the smallest dose (100 mg./Kg.). The highest dose given (500 mg./Kg.) was in the toxic range, and, as indicated by the standard errors in the table, variation in excretion was greater. Nevertheless, the results were generally consistent with those obtained at other dose levels.

Excretion was essentially complete within twenty-four hours after the oral administration of 100 and 250 mg./Kg., but, at 500 mg./Kg., considerable amounts appeared in the urine during the second day.

The sex difference observed with single intravenous doses was also apparent with single oral doses, especially during the first twenty-four hours following drug administration. Age and weight differences were excluded as contributing factors by comparing properly selected groups within the large group.

Excretion with Repeated Oral Doses to Rats.—Decapryn Succinate was administered orally to rats once a day for periods of four or eleven days in two different experiments employing several dose levels. It is seen from Table II that excretion tended to increase during the first few days of the experiment, but there was considerable irregular variation. In two instances, excretion remained at quite a high level on the day after the cessation of

TABLE I.—URINARY EXCRETION OF DECAPRYN FOLLOWING THE ADMINISTRATION OF SINGLE DOSES OF DECAPRYN SUCCINATE TO RATS

Route of Administration	Sex	Dose, Mg./Kg.	Decapryn Excretion in Urine: 1st 24 Hr. No. of Animals	1st 24 Hr. Mean and Standard Error, % of Dose	2nd 24 Hr. No. of Animals	2nd 24 Hr. Mean and Standard Error, % of Dose
I. V.	M	25	6	7.6 ± 1.3	6	0.9 ± 0.6
I. V.	F	25	6	16.9 ± 2.7	6	0.5 ± 0.3
Oral	M	100	10	8.1 ± 1.2	3	0.1 ± 0.1
Oral	F	100	16	17.5 ± 1.5	3	0.6 ± 0.1
Oral	M	250	8	14.4 ± 1.9	2	0.9 ± 0.5
Oral	F	250	10	23.1 ± 2.9	2	1.4 ± 1.4
Oral	M	500	6	14.3 ± 2.5	3	7.0 ± 1.7
Oral	F	500	6	24.7 ± 5.0	3	11.0 ± 0.6

TABLE II.—URINARY EXCRETION OF DECAPRYN WITH REPEATED DAILY ORAL DOSES OF DECAPRYN SUCCINATE TO FEMALE RATS

Daily Dose, Mg./Kg.	Per Cent of Daily Dose Excreted in Urine, Days: 1	2	3	4	5	6	7	8	9	10	11	12	13
100	9.3	25.2	19.3	*33.3*[a]	0.7	1.0	..	..	..	..	..	..	..
250	16.7	21.9	30.8	*32.3*	23.4	7.1	..	..	..	..	..	..	..
250	12.2	26.6	30.1	*25.7*	17.0	1.9	..	..	..	..	..	..	..
25	1.8	12.8	8.0	8.0	27.2	9.6	8.0	3.2	4.8	4.8	*3.2*	0	0
25	2.9	11.4	10.0	11.4	20.0	22.9	11.4	7.1	8.6	8.6	*8.6*	0	0
250	16.2	33.5	31.3	28.3	37.9	34.1	40.9	18.3	49.7	43.4	*24.9*	2.4	0
250	26.3	30.5	35.5	25.3	41.0	42.8	42.8	41.0	46.4	28.2	*21.7*	6.5	1.9

[a] Italicized figures indicate last day of drug administration.

drug administration, and, in one of these, an amount equivalent to 7.1% of the daily dose was excreted on the next day. In other animals, particularly on the lower doses, the urinary excretion of the amine stopped almost entirely within twenty-four hours after drug administration was discontinued.

Excretion with Repeated Oral Doses to Dogs.—Table III gives the results of studies with dogs

TABLE III.—URINARY EXCRETION OF DECAPRYN WITH REPEATED ORAL DOSES OF DECAPRYN SUCCINATE IN DOGS

No. of Animals	No. of Samples	Dose, Mg./Kg. t.i.d.	No. of Days on Test	Mean and Standard Error of Decapryn Excretion, % of Dose
4	8	3.0	36–46	18.3 ± 1.6
4	8	7.5	30–50	17.1 ± 1.5

which had received Decapryn Succinate three times a day for thirty to fifty days. The average twenty-four-hour excretion value, expressed as Decapryn Succinate, for 4 control dogs was 4.4 ± 1.3 mg. This mean value was subtracted from the figures for experimental dogs before calculating the percentage excretion.

On both of the dose levels studied, the excretion amounted to about 18% of the dose and was thus of the same order of magnitude as that found for rats receiving moderate oral doses. However, there was no difference in excretion levels of male and female dogs, and the values are combined in the table.

Identification of the Material Excreted by Rats.—Since the colorimetric method used is not specific, several studies were carried out in an attempt further to characterize the excretory product. Several batches of composite urine samples, from rats which received Decapryn Succinate, were employed as starting material. The samples were made alkaline and extracted with toluene. The toluene was then extracted with dilute hydrochloric acid, and the resulting water-clear solution was used in subsequent tests. On the basis of colorimetric assays, these extractions were performed without loss of chromogenic material.

Ultraviolet Absorption.—An aqueous sample was made alkaline and extracted with chloroform; a portion of this extract was diluted to contain the colorimetric equivalent of 4 mg. of Decapryn base/100 cc. The ultraviolet absorption curve for this solution and that for a standard solution containing 4 mg. of Decapryn base/100 cc. of chloroform were determined with a Beckman spectrophotometer. The marked similarity of these curves, shown in Fig. 1, constitutes presumptive evidence that the excreted material consists largely of unchanged Decapryn.

Isolation of the Hydrochloride.—A chloroform extract of the aqueous solution was evaporated to dryness, and the residue was taken up in petroleum ether. To this solution was added a small volume of a solution of dry hydrogen chloride in petroleum ether. The resulting white precipitate was filtered, washed with petroleum ether, and dried. This material was recrystallized three times from isopropanol. The purified product had a melting point of 160–162° (uncorr.). A known sample of Decapryn hydrochloride melted at 162–164° (uncorr.), and the melting point of a mixture of the two was 159–160° (uncorr.). This demonstrates further that Decapryn base was responsible for at least a part of the color produced in the assay.

Precipitation of the Reineckate.—The insolubility of many amine reineckates suggested an additional possibility for the study of the excreted material. The conditions used for precipitation were those described by Rennick, *et al.* (10), for the determination of the tetraethylammonium ion. A 2% solution of Reinecke salt in methanol was added to an aqueous solution of Decapryn Succinate, and precipitation was completed by refrigeration. The average weight of dry precipitate was 1.5 mg./mg. of Decapryn Succinate used; this is the theoretical value for the formation of Decapryn monoreineckate.

It was observed further that, when these precipitates were suspended in hot water, made alkaline with sodium hydroxide, and extracted with toluene,

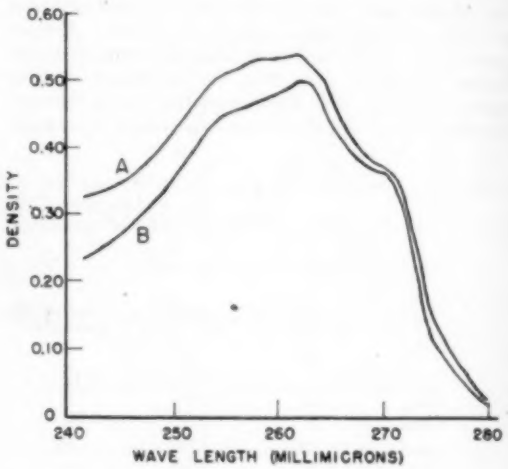

Fig. 1.—Ultraviolet absorption curves of Decapryn solutions.

Curve A: 4 mg. of Decapryn base dissolved in 100 cc. of chloroform. *Curve B:* Urine extract containing the colorimetric equivalent of 4 mg. of Decapryn base in 100 cc. of chloroform.

colorimetric assay gave reasonably satisfactory recovery (94 to 104%) of the initial quantity of Decapryn Succinate.

This procedure was consequently applied to extracts of pooled rat urine. The weights of the precipitates, converted to terms of Decapryn Succinate on the basis of monoreineckate formation, corresponded to recoveries of 95–97% of the material shown to be present by the colorimetric method.

Acetone solutions of the precipitated reineckates were prepared, and color intensities were determined in the Fisher Electrophotometer with a 525 mμ filter. The data showed that samples obtained from Decapryn Succinate and from urine extracts were identical in this respect.

Portions of the precipitates, without further purification, were subjected to elementary analysis. The results (Table IV) show only that neither substance was chemically pure.

Protection Against Lethal Effects of Histamine.—Several of the aqueous extracts of urine were in-

jected intravenously into guinea pigs and the degree of protection afforded against the lethal effects of intravenously administered histamine was determined. Concentrations of the solutions used were based on the values obtained from colorimetric assay, and it was found that an amount equivalent, colorimetrically, to 2 mg. of Decapryn Succinate/Kg. protected against as many as 7 fatal doses of histamine, while the equivalent of 16 mg./Kg. protected against as may as 60 fatal doses. It is apparent that the excreted material has a rather potent antihistaminic action.

TABLE IV.—ELEMENTAL ANALYSIS OF DECAPRYN REINECKATE PRECIPITATES

Source of Precipitate	Composition, % C	H	N	Cr
Decapryn Succinate	43.22	5.30	17.66	8.90
Urine extract	35.71	6.50	18.74	10.80
Theoretical for Decapryn Monoreineckate	42.84	4.78	19.04	8.83

Decapryn Succinate in Tissues.—Application of the colorimetric method demonstrated that no trace of chromogenic amine was present in the blood, liver, spleen, heart, lungs, kidneys, or skeletal muscle of control rats, or in the blood of control rabbits and dogs. The same tissues of rats also showed no trace of amine twenty-four hours after they received single oral doses of 100 mg. of Decapryn Succinate/Kg. Other assays demonstrated the absence of amine in the blood (*a*) of rats one hour after the oral administration of 25 mg. of Decapryn Succinate/Kg., (*b*) of rabbits one hour after the intravenous administration of 20 mg./Kg., and (*c*) of dogs thirty-six hours after the termination of an experiment in which a dose of 7.5 mg./Kg. was administered orally three times daily for about eight weeks.

SUMMARY

1. Average urinary excretion values ranged, depending on the size of the dose, between 7 and 21 per cent of the dose for male rats and between 17 and 36 per cent for females following the administration of single intravenous and oral doses of Decapryn Succinate. Excretion was virtually complete within twenty-four hours following doses of 250 mg./Kg. or less.

2. Excretion by rats tended to increase during the first few days of repeated daily oral administration, and it dropped sharply after drug administration was discontinued. Dogs receiving daily oral doses of Decapryn Succinate for prolonged periods consistently eliminated roughly 20 per cent of the daily dose in the urine, and there was no difference in excretion between the sexes.

3. No trace of amine was found in the tissues of rats twenty-four hours after the oral administration of 100 mg. of Decapryn Succinate/Kg., or in the blood under any of the conditions employed.

4. The probable identity of the excreted material and Decapryn has been established by (*a*) ultraviolet absorption studies, (*b*) isolation of Decapryn hydrochloride, (*c*) precipitation of the reineckate, and (*d*) measurement of protection against the lethal effects of histamine.

5. It is concluded that the bulk of the administered Decapryn Succinate is destroyed in the body.

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WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

3,5-Diiodothyronine
D-Galactoascorbic acid
L-Glucoascorbic acid
Rubrophen
Hypoxanthine desoxyribose phosphoric acid
Fructose-6-phosphate
Kellin
Hydnocarpic acid
Petroselinic acid
Isopelletierine
D-Lupanine hydrochloride
Tigogenin
Betulin
Phenol sulfatase

The Antibacterial Actions of Quaternary Ammonium Sulfonamides*

By C. A. LAWRENCE and G. R. GOETCHIUS,† with the Technical Assistance of J. F. GAIN

The results of a study of the antibacterial actions of a number of quaternary ammonium sulfonamides are reported. The antibacterial actions of these compounds have been found to be due to the quaternary ammonium component in the molecule and not to the sulfonamide radical.

THE DESIRABILITY of having available a single compound which exhibits not only sulfonamide action but bactericidal, surface-acting properties as well, has been suggested by Barry and Puetzer (1). These workers implied that a compound containing a highly germicidal quaternary ammonium residue as the surface-active portion of the molecule, combined with a sulfonamide group, may be useful in nasal therapy and for other chemotherapeutic applications. The chemistry of several of these compounds, namely the cetyltrimethyl ammonium, cetylpyridinium, and laurylpyridinium salts of one or more of the commonly known sulfonamides, was described by the workers mentioned. They concluded from their studies that the compounds were highly bactericidal against *Staphylococcus aureus* and *Escherichia coli, in vitro*. In some instances, the compounds were more effective than the corresponding ammonium components contained in the molecule.

Studies in our laboratories have indicated several other quaternary ammonium sulfonamides are likewise effective in high dilutions in destroying a variety of pathogenic bacteria. Unlike the compounds described by Barry and Puetzer in which the sulfonamide portion of the molecule is the anion, our studies involved the use of compounds in which the sulfonamide radical is part of the cation. The difference in the structural configuration of the two types of quaternary ammonium sulfonamides, as well as the compounds used in the present study, are shown in Figs. 1 and 2. The chemistry of the latter will be described elsewhere by Dr. E. D. Homiller of these laboratories.

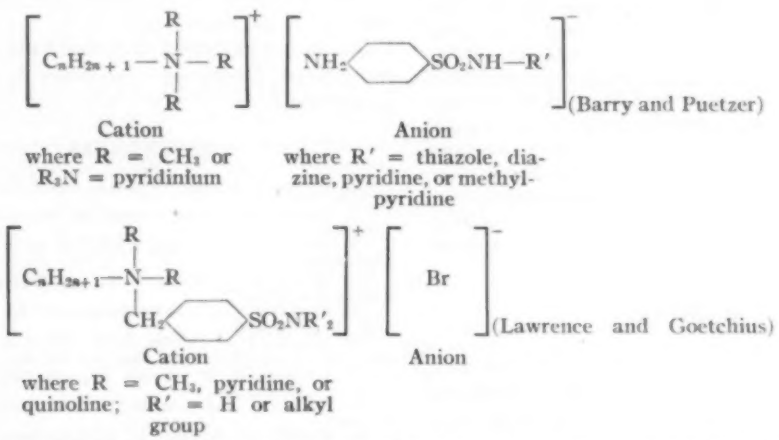
Cation
where R = CH3 or R3N = pyridinium
Anion
where R′ = thiazole, diazine, pyridine, or methylpyridine
(Barry and Puetzer)
Cation
where R = CH3, pyridine, or quinoline; R′ = H or alkyl group
Anion
(Lawrence and Goetchius)

Fig. 1.—Structural formulae of the two types of quaternary ammonium sulfonamides.

EXPERIMENTAL

Phenol Coefficient.—The Food and Drug Administration (F. D. A.) method for determining phenol coefficients (2) was one means of evaluating the quaternary ammonium sulfonamides. *S. aureus* (No. 209) and *Eberthella typhosa* (Hopkins strain) were used as the test organisms. The seeding tubes containing the dilutions of the compounds were placed in a water bath at 20° for five minutes or

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until the temperature of the bath was reached, before the addition of the test culture. The tubes of F. D. A. broth containing transplants from the seeding tubes were incubated at 37° for forty-eight hours and observed for the presence or absence of visible growth. Tubes showing no growth were tested for bacteriostasis by transferring a quantity (three 4-mm. loopfuls) of the broth mixture to additional tubes containing sterile broth, as outlined in the F. D. A. procedure. The phenol coefficient values on duplicate tests and the highest dilution of the quaternary ammonium sulfonamide which destroyed the test organisms in ten but not in five minutes are given in Table I.

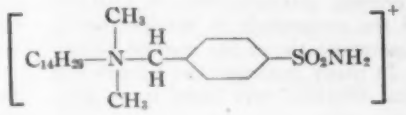
The results of this study show the most active

TABLE I.—PHENOL COEFFICIENTS ON QUATERNARY AMMONIUM SULFONAMIDES

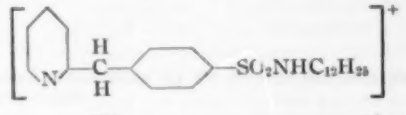
Compound	*S. aureus* Phenol Coefficient	*S. aureus* Limiting Dilution Effective—10 Min.	*E. typhosa* Phenol Coefficient	*E. typhosa* Limiting Dilution Effective—10 Min.
1	233	12,000	267	24,000
2	233	12,000	267	24,000
3	<16[a]	<1,000	<11	<1,000
4	83	5,000	50	5,000
5	<16	<1,000	<11	<1,000
6	<16	<1,000	<11	<1,000

[a] <16 = No phenol coefficient value obtainable since highest concentration tested (1:1000) failed to destroy the test organism in ten minutes.

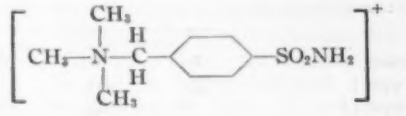
Compound 1
4-Sulfonamidobenzyltetradecyldimethylammonium bromide—mol. wt. 491.5



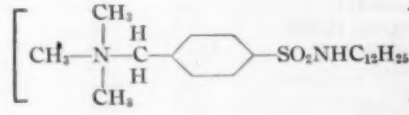
Compound 2
4 - Dodecylsulfonamidobenzylpyridinium bromide—mol. wt. 497.5



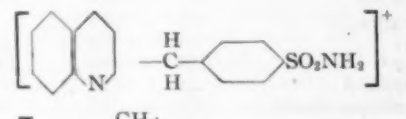
Compound 3
4-Sulfonamidobenzyltrimethylammonium bromide—mol. wt. 309.2



Compound 4
4-Dodecylsulfonamidobenzyltrimethylammonium bromide—mol. wt. 379.3



Compound 5
4-Sulfonamidobenzylquinolinium bromide—mol. wt. 379.3



Compound 6
4-Dodecylsulfonamidobenzyldimethyldodecylammonium bromide—mol. wt. 631.8

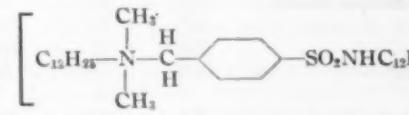


Fig. 2.—Structural formulae of quaternary ammonium sulfonamides.

compounds are No. 1 (4-sulfonamidobenzyltetradecyldimethylammonium bromide) and No. 2 (4-dodecylsulfonamidobenzylpyridinium bromide). Identical phenol coefficient values were obtained on the latter salts, namely 233 and 267, against *S. aureus* and *E. typhosa*, respectively. Considerably lower phenol coefficient values were obtained on compound No. 4 (4-dodecylsulfonamidobenzyltrimethylammonium bromide) while the remaining quaternary ammonium sulfonamides failed to show evidence of a germicidal action in the highest concentration tested (1:1000 or 0.1%).

Bactericidal and Bacteriostatic Tests.—The compounds were tested against a variety of bacteria in the presence of a fluid medium by dissolving them directly in broth in a 1:1000 concentration. From this initial concentration, serial dilutions were made in broth up to and including 1:16,384,000. The compounds were all completely soluble in broth in the effective dilutions with the exception of No. 6 (4 - dodecylsulfonamidobenzyldimethyldodecylammonium bromide) which contained some undissolved material in the higher concentrations. The drug-broth solutions were distributed in 5-ml. quantities in test tubes and autoclaved at ten pounds for ten minutes. The inoculum consisted of one 4-mm. loopful of a twenty-four-hour broth culture of the test organism. A number of Gram-negative bacilli representative of the colon-typhoid-dysentery group were tested in the presence of beef extract broth (F. D. A.). The latter medium was also used for *Pseudomonas aeruginosa*, *Vibrio cholerae*, and *S. aureus*. Veal infusion-glucose broth containing 1% normal horse serum was used in tests in which Types I, II, and III pneumococci and *Streptococcus pyogenes* (C-203) were studied. This same medium, but without serum, was selected for studying the other streptococci. The series was completed with tests on the *Brucella* in tryptose-phosphate broth containing 0.1% agar, and four anaerobic clostridia in fluid thioglycollate medium.

The inoculated media were incubated at 37° and

observed for the presence of visible growth at the end of twenty-four, forty-eight, and seventy-two hours. Failure of growth to appear, or to be less than half that in the drug-free control, after the initial twenty-four-hour incubation period was considered evidence of bacteriostasis. Tubes showing no growth at the end of seventy-two hours were tested for bactericidal action by transferring three 4-mm. loopfuls of the test mixture to a second tube of liquid medium. Lack of growth in the subculture tubes following seventy-two hours' incubation was taken as evidence of a bactericidal action of the compound upon the test organisms in the original tube.

The high antibacterial activity of compounds 1 and 2 is again evident from the data presented in Table II. In general, concentrations of 1:64,000 to 1:256,000 of the compounds in nutrient media proved to be bacteriostatic to the various Gram-positive cocci. In many instances the bactericidal and bacteriostatic dilutions were found to be identical.

Of the four strains of clostridia, *Cl. histolyticum* was found to be the most resistant organism to the lethal action of the quaternary ammonium compounds. Number 1 was found to be completely ineffective against the organism, whereas concentrations of 1:4000 to 1:16,000 of some of the other compounds were required to inhibit its growth.

Inspection of the data (Table II) will, furthermore, reveal compound 4 is somewhat less active than Nos. 1 and 2 against most of the organisms studied. Also, while No. 6 is entirely ineffective as a germicidal agent against all the Gram-negative bacteria, it compared in activity with compound 4 against many of the Gram-positive cocci. Although not indicated in the table, compounds 3 (4-sulfonamidobenzyltrimethylammonium bromide) and 5 (4-sulfonamidobenzylquinolinium bromide) were found to be entirely inactive to all of the organisms studied.

Surface tension.—Gershenfeld and Milanick (3), Baker, Harrison, and Miller (4), and Rawlings, Sweet, and Joslyn (5) have shown that while many

TABLE II.—LIMITING DILUTIONS OF QUATERNARY AMMONIUM SULFONAMIDES SHOWING ANTIBACTERIAL ACTIVITY

	Compound							
	1		2		4		6	
Organism	Bs	Bc	Bs	Bc	Bs	Bc	Bs	Bc
Pneumococcus Type I	256	64	512	256	128	32	1024	32
Pneumococcus Type II	64	32	256	128	32	32	64	16
Pneumococcus Type III	64	64	256	256	64	64	256	256
Streptococcus pyogenes (C203)	512	256	512	128	256	64	1024	64
Streptococcus viridans	1024	512	4096	2048	512	256	256	16
Streptococcus fecalis	128	64	512	16	64	16	128	2
Streptococcus mitis	256	256	1024	1024	128	64	256	2
Staphylococcus aureus	512	128	512	256	512	64	128	<1
Pseudomonas aeruginosa	<1	<1	1	<1	1	<1	<1	<1
Escherichia coli	64	32	64	16	32	16	<1	<1
Eberthella typhosa	128	64	256	64	32	16	<1	<1
Salmonella paratyphi A	128	32	64	8	16	8	<1	<1
Shigella paradysenteriae Sonne	128	64	64	64	64	32	<1	<1
Vibrio cholerae	128	64	256	128	128	1	<1	<1
Brucella abortus	256	32	512	16	64	32	16	<1
Brucella melitensis	512	64	64	32	256	64	4	<1
Brucella suis	8	4	8	8	32	8	4	<1
Clostridium welchii	128	32	128	64	64	32	8	2
Clostridium tetani	128	64	128	64	256	64	8	2
Clostridium histolyticum	<1	<1	16	4	16	4	8	<1
Clostridium oedematiens	32	16	32	16	32	16	8	8

Bs = Bacteriostatic.
Bc = Bactericidal.
All values given represent dilutions in thousands, i.e., 256 = 256,000, etc.
Figures preceded by sign < indicate the highest concentration tested failed either to kill and, or, inhibit the growth of the test organism.

With but one exception, the Gram-negative bacilli were found to be destroyed by relatively low concentrations of compounds 1 and 2. In most instances, the bacteriostatic dilutions were but one tube removed in the serial dilutions showing a bactericidal effect on the same organism. Both compounds, as well as Nos. 4 and 6, were found to have little, if any, effect on the culture of *Ps. aeruginosa*.

A definite degree of resistance to the antibacterial actions of the quaternary ammonium sulfonamides was exhibited by *Brucella suis*. The *Br. abortus* and *Br. melitensis* strains, on the other hand, were inhibited in their growth and destroyed by relatively high dilutions of compounds 1, 2, and 4.

of the more effective cationic detergents have low surface tension, some of the less active ones are equally surface active. Furthermore, in many cases the anionic compounds of feeble germicidal action have an effect on surface tension comparable to the most active cationic detergent.

In the present study, the surface tensions of 0.01% concentrations of the quaternary ammonium sulfonamides were determined by the "ring method" with a du Nouy tensiometer. Readings were made soon after the solutions were prepared and again after allowing them to stand at room temperature for twenty-four hours. The values, corrected for temperature, are given in Table III. Included in

the table are surface-tension readings on 0.01% aqueous and broth solutions of compounds 3 and 5 in the presence of 0.1% of a nonionic detergent.[1]

From the data (Table III), it will be noted that compounds 3 and 5, which were ineffective as antibacterial agents, were correspondingly inactive as surface-tension depressants in water and nutrient broth. The low surface-tension environment is not the only factor responsible for the high germicidal activity of several of the other quaternary ammonium sulfonamides as evidenced from the surface-tension values obtained on compound 6. This compound is the most active surface-tension depressant in the series studied, yet it failed to give a phenol coefficient value against *S. aureus* and *E. typhosa* (Table I) and was, furthermore, less active than compounds 1, 2, and 4 when tested in the presence of a nutrient medium (Table II).

TABLE III.—SURFACE TENSIONS OF AQUEOUS AND BROTH SOLUTIONS OF QUATERNARY AMMONIUM SULFONAMIDES

Compound Number	Surface Tension of 0.01% Solutions: Distilled Water	F. D. A. Broth
1	59.7	42.7
2	56.8	38.4
3	75.2	53.4
4	58.5	38.9
5	75.3	53.7
6	31.4	32.2
3 (+ 0.1% nonionic detergent)	35.8	36.4
5 (+ 0.1% nonionic detergent)	37.0	37.6
Controls		
Distilled water	75.3	..
F. D. A. Broth	..	53.7
0.1% nonionic detergent	34.5	..

The resulting solutions, with surface tensions of 36.4 and 37.6, respectively (Table III), were incapable of inhibiting the growth of test organisms. Our studies, furthermore, indicated the nonionic detergent and the active quaternary ammonium sulfonamides were compatible with respect to the antibacterial actions of the latter.

Germicide Inactivation.—Since the quaternary ammonium sulfonamides contain two possible antibacterial components, a quaternary ammonium and a sulfonamide group, it appeared desirable to determine whether the bactericidal and bacteriostatic activities could be attributed to all, or part, of one of these components in the molecule. A test was designed, therefore, to include a suitable quaternary inactivator and a sulfonamide inactivator in the medium in which the more active quaternary ammonium sulfonamides were tested against a representative culture of the Gram-positive, as well as the Gram-negative, group of bacteria.

Compounds 1 and 2 were dissolved primarily in distilled water to a concentration of 1:200. Serial dilutions were prepared in F. D. A. broth in a range from 1:2000 up to and including 1:256,000. To 4 ml. of the drug dilution was added either 1 ml. of 1:5000 para-aminobenzoic acid (sulfonamide inactivator), 1 ml. of 1% Suramin Sodium (6), U. S. P. XIII (quaternary ammonium inactivator), or 1 ml. of distilled water (control). One series of tubes was inoculated with a 4-mm. loopful of a twenty-four-hour broth culture of *S. aureus* while another series received an inoculum of *E. typhosa*. The tubes were incubated at 37° for seventy-two hours and the presence or absence of growth recorded at this time. Tubes showing no growth at the seventy-two-hour period were tested for bacteriostasis by subculturing three 4-mm. loopfuls of the test suspension to 10 ml. of plain F. D. A. broth, as well as to F. D. A. broth containing Suramin Sodium. The latter compound was also added directly to the original medication tubes showing no visible growth at the seventy-two-hour observation period. Lack of growth in the subculture tubes, following seventy-two hours' incubation, was considered evidence of a bactericidal action of the quaternary ammonium sulfonamides upon the test organisms in the original medication tubes. Since the results of the test on the two quaternary ammonium compounds were comparable, the data obtained on only one of these are presented in Table IV.

TABLE IV.—INACTIVATION OF QUATERNARY AMMONIUM SULFONAMIDES[a]

	S. aureus												*E. typhosa*											
	Control				PABA				Suramin				Control				PABA				Suramin			
Dilution	1[a]	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1:2,000	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	+	+	+	+
1:4,000	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	+	+	+	+
1:8,000	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	+	+	+	+
1:16,000	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	+	+	+	+
1:32,000	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	+	+	+	+
1:64,000	–	+	+	+	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:128,000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:256,000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

[a] 1 = Original medication tube; 2 = Medication tube + Suramin Sodium added at seventy-two-hour period; 3 = F D A broth subculture tube; 4 = F D A broth + Suramin Sodium subculture tube; – = No visible growth; + = Growth.

The relationship of surface tension to the antibacterial action was further investigated in the following manner: The nonionic detergent of low surface tension, which in itself was devoid of any demonstrable antibacterial action, was incorporated in a medium containing 0.01% of compounds 3 and 5.

It is evident from the data given in the table that

[1] One of a series of viscous, water-soluble nonionic agents which are described as long-chain fatty acid esters containing multiple ether linkages, known as Intral 224, a Synthetic Chemical, Inc., product.

the activity of the quaternary ammonium sulfonamides can be attributed to the quaternary component in the molecule. The antibacterial activity could be completely neutralized by Suramin Sodium, whereas para-aminobenzoic acid failed to have any adverse action on the germicidal action of the compounds in the dilutions tested.

The data furthermore reveal that the quaternary ammonium compounds possess a definite bactericidal action in relatively high dilutions, since in most instances the organisms could not be "revived" upon the addition of Suramin Sodium to the original "control" and "PABA" series at the seventy-two-hour period.

SUMMARY

Certain salient features to be noted from a comparison of the structural configuration to the antibacterial activities of several quaternary ammonium sulfonamides may be listed as follows:

1. The addition of a long-chain alkyl group on the quaternary nitrogen of 4-sulfonamidobenzyltrimethylammonium bromide will result in a compound with high antibacterial and surface-tension depressant actions.

2. Substitution of a long-chain alkyl group on the sulfonamide nitrogen of 4-sulfonamidobenzyltrimethylammonium bromide will also result in a compound with considerable activity.

3. A compound containing a long-chain alkyl group on both the quaternary and sulfonamide nitrogens of 4-sulfonamidobenzyltrimethylammonium bromide has a low surface tension, but its germicidal effects are less marked than those of the single alkyl substituted derivatives.

4. The antibacterial actions of the quaternary ammonium compounds described in this study are due to the quaternary ammonium component in the molecule and not the sulfonamide radical.

Zienty (7), in a recent publication appearing after the present study had been completed, described a large series of quaternary ammonium salts of pyridine-3-carboxamides and N'-substituted pyridine-3-sulfonamides. A number of the latter compounds compared favorably with the germicidal activity of benzalkonium chloride U. S. P. XIII.

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1949 Iodine Research Award Nominations Requested

Nominations are now being received by the AMERICAN PHARMACEUTICAL ASSOCIATION for the 1949 Iodine Educational Bureau Award recognizing outstanding research in the chemistry and pharmacy of iodine and its compounds as applied in pharmacy or medicine. Any member of the ASSOCIATION may propose a nominee by submitting eight copies of each of the publications to be considered in the competition, a biographical sketch of the nominee including date of birth, and a list of his publications. Eight copies of the nomination must be submitted to the Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION, Dr. Robert P. Fischelis, 2215 Constitution Ave., N. W., Washington 7, D. C. To be eligible for the 1949 Award, nominations must be received before January 1.

Establishment of the iodine award was first announced at the Milwaukee convention of the AMERICAN PHARMACEUTICAL ASSOCIATION which administers the competition.

A nominee must be a resident of the United States or Canada. He must have accomplished outstanding research in the chemistry or pharmacy of iodine and its compounds as applied in pharmacy or medicine.

During the period covered by the nomination the nominee shall have been actively engaged in, shall have completed, or shall have published a report upon the line of investigation for which the award is made. During a period of two years prior to the date of nomination, the nominee shall not have been engaged in research under the sponsorship of the Iodine Educational Bureau, Inc.

The award consists of $1000 and a diploma setting forth the reasons for selection of the recipient. It may be presented annually at the annual meeting of the AMERICAN PHARMACEUTICAL ASSOCIATION.

The recipient will deliver a paper or lecture upon the subject of his scientific work at the meeting at which the award is conferred. His paper, or address, will then be published in the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION. In addition to the sum of the award, the recipient will receive an allowance of not more than $250 to defray his expenses in attending the meeting.

The recipient will be selected by an award committee which is appointed by the chairman of the ASSOCIATION's Council and which functions under prescribed rules.

Should the award committee fail to find a suitable recipient in any year, two awards may be made during the next year if suitable recipients are selected.

Studies on Alcohol-Soluble Fungistatic and Fungicidal Compounds. III. Evaluation of the Antifungal Properties of Quinones and Quinolines*

By KURT A. OSTER and MILTON J. GOLDEN†

Ten commonly used antifungal compounds have been found to possess only relatively weak fungistatic and fungicidal properties. In a search for more potent therapeutic substances fifty-five chemicals belonging to the quinone and quinoline series were subjected to a preliminary screening test, and twelve of these were subsequently subjected to more intensive *in vitro* studies. Four compounds of the series were found to possess outstanding antifungal properties. The potency of one chemical was as much as seventy-seven times greater than that of the average substances now in therapeutic usage.

In two previous publications (1, 2) the currently used laboratory methods for evaluating fungistatic and fungicidal compounds were subjected to a critical study. The necessity for modifying these tests was proved in order for them to be regarded as significant and to permit comparison of true potency of antifungal substances. A new expression of fungistasis, the activity coefficient, was introduced at this time and the fungicidal tests were made more stringent. Use of these methods, thus modified and improved, demonstrated that ten compounds now popularly used in the treatment of dermatophytosis possess only relatively weak fungistatic and fungicidal properties.

This finding prompted the search for more specific antifungal compounds, the potencies of which could be measured by the improved laboratory test methods and which, after screening out the less active substances, could be subjected to more intensive laboratory studies and finally to clinical trials. This investigation is concerned with the laboratory testing of compounds belonging to the quinone and quinoline series, some of which are known to exert certain antifungal action.

Reports that certain quinones are bactericidal have been forthcoming for the last forty years (3). The fungicidal properties of quinones was the subject of a patent issued in 1926 to Brodersen and Ext (4). Tetrachloro-*p*-benzoquinone was patented as a fungicide by Ter Horst (5). The same investigator described the fungicidal properties of 2,3-dichloro-1,4-naphthoquinone for agricultural and textile uses in 1943 (5). Gonzalez (6) reported the inhibitory effect of quinones on the growth of *Penicillium notatum*. Colwell and McCall (7) investigated the mechanism of bacterial and fungus growth inhibition by 2-methyl-1,4-naphthoquinone, suggesting that its antibacterial and antifungal effects are attained by blocking sulfhydryl-containing enzymes or growth metabolites, leaving open, however, the possibility of different mechanisms.

In 1947, Ball, Anfinsen, and Cooper (8), investigating the inhibition of respiratory processes by naphthoquinones, found that the activity of these compounds depends upon the nature of the alkyl group and suggested that the inhibitory action results from a combination of the naphthoquinones with an unknown enzyme which mediates the reaction between cytochrome *c* and cytochrome *b*. Hoffman-Ostenhof (9) stated that the antibiotic action of quinones is due not to a single reaction, but to a complex mechanism. The mode of action proposed by Colwell and McCall (7) would be only one of the means by which the quinones exert their effects and would not be the dominant one in most cases.

8-Hydroxyquinoline, first introduced in Germany in 1895 as an "antiseptic and disinfectant," has long been used as a bactericidal and fungicidal agent in many medical preparations. Its fungistatic action was explained by Zentmyer (10) as being due to "the phenomenon of forming inner complex salts with metal ions and thus rendering them unavailable to microorganisms." The same hypothesis is under investigation by Albert and his collaborators (11). Rigler and Greathouse (12) studied the fungicidal potencies of quinoline homologs and derivatives against *Phymatotrichum omnivorum*, a fungus causing cotton root disease, and found 8-hydroxyquinoline to be over six hundred times more potent than the 2-isomer.

The antifungal action of quinones and quinolines has not yet been studied intensively on that group of fungi which causes human dermatophytosis, as far as our study of the pertinent literature has revealed.

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METHODS

Laboratory methods employed in the evaluating of the quinone and quinoline compounds have been described in detail in two previous publications (1, 2). For determining fungistasis, methods described by Burlingame and Reddish (13) and by Schamberg and Kolmer (14) were utilized. Fungicidal properties of the chemicals were measured by a modification (2) of the original Burlingame and Reddish technique. Ten-day-old cultures of *Trichophyton mentagrophytes* (No. 640 Emmons) served as test organism throughout the entire investigation. All substances were dissolved in 95% ethyl alcohol, if their solubilities so permitted. A few compounds, insoluble in alcohol in the desired concentration, were dissolved in acetone. The reported results represent the average value of at least three separate determinations. All cultures were incubated at 28° C ± 1°.

EXPERIMENTAL

Eleven derivatives of benzoquinone, twenty derivatives of naphthoquinone and twenty-four quinoline derivatives were evaluated. These fifty-five compounds were first subjected to a screening test for the purpose of eliminating those which were weak in fungistatic activity. The screening procedure consisted of measuring the radius segment of the cleared zone of growth inhibition around the solution cup according to the method of Burlingame and Reddish (13), using in each case a 0.1% solution of the test compound in 95% ethyl alcohol for the evaluation. Tables I, II, and III list the compounds tested and the results obtained in millimeters clearance on seeded Sabouraud's maltose agar plates.

Dosage-response curves were obtained with various concentrations of the twelve selected test substances, using the Burlingame and Reddish test method (Fig. 1). Activity coefficients of the compounds were calculated from these curves by dividing the interpolated concentration which effects a 5-mm. radius segment of clearance into the figure one, as was suggested by Oster and Golden (1).

The same group of twelve compounds was also tested for fungistatic activity by the method of Schamberg and Kolmer (14), employing the procedure of incorporating various dilutions of the test compound in Sabouraud's maltose agar. Table IV lists the maximum dilutions effecting complete fungistasis with this technique and the activity coefficients calculated from the dosage-response curves of Fig. 1.

Fungicidal action of the investigated compounds was tested according to the authors' modification of the Burlingame and Reddish technique (2). The severe criterion of a one-minute contact time of the test substance with the test fungus was again used in this evaluation. The age of the culture was at least ten days, and the medium contained 10% serum. Table V lists the minimum fungicidal concentrations of ten of the twelve investigated compounds. To arrive at these values at least two out of three determinations for each substance had to be proved fungicidal.

RESULTS

Of the eleven representatives of the benzoquinone group listed in Table I, two compounds, *p*-xyloquinone and *p*-thymoquinone, with methyl substitutions in the 2 position and methyl and isopropyl groups, respectively, in the 5 position, exhibited highly fungistatic properties. A single methyl group in the 2 position, as in the case of *p*-toluquinone,

TABLE I.—INFLUENCE OF VARIOUS SIDE GROUPS ON THE FUNGISTATIC ACTIVITIES OF ELEVEN BENZOQUINONES (CONCENTRATION 0.1%, SOLVENT 95% ETHYL ALCOHOL)

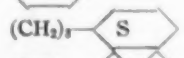
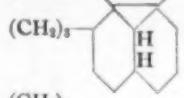
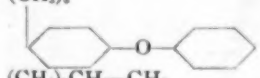
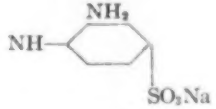
Compound	Positions on the Ring 1	2	3	4	5	6	Clearance[a] Mm.
p-Quinone	O	H	H	O	H	H	6
Hydroquinone	OH	H	H	OH	H	H	None
p-Toluquinone	O	CH_3	H	O	H	H	11
Toluhydroquinone	OH	CH_3	H	OH	H	H	4
p-Phenylquinone	O	C_6H_5	H	O	H	H	8
p-Xyloquinone	O	CH_3	H	O	CH_3	H	19
p-Thymoquinone	O	CH_3	H	O	CH (CH_3, CH_3)	H	18
2,5-Ditertiary butyl quinone	O	C (CH_3, CH_3, CH_2)—CH_3	H	O	C (CH_3, CH_3, CH_2)—CH_3	H	4
Rapanone	O	$(CH_2)_{12}CH_3$	OH	O	H	OH	None
2,6-Dichloroquinone	O	Cl	H	O	H	Cl	3
Chloranil (dissolved in acetone)	O	Cl	Cl	O	Cl	Cl	4

[a] Cleared zone of growth inhibition around solution cup in agar plate, measured in millimeters as radius segment.

Twelve compounds of the entire series of fifty-five were selected for further study. Of these twelve, six were chosen for their demonstrably high fungistatic activity and six for their structural nature in the hope of gaining information as to the mechanism of the strong antifungal action of the first six compounds.

showed less activity. Unsubstituted *p*-quinone exhibited low activity; as hydroquinone, its reduced form, it showed no activity whatever at the test concentration. The activity of *p*-toluquinone was also curtailed by reduction to the corresponding hydroquinone. Considering *p*-quinone as the basic value, a phenyl group in the 2 position slightly in-

TABLE II.—INFLUENCE OF VARIOUS SIDE GROUPS ON THE FUNGISTATIC ACTIVITIES OF TWENTY NAPHTHOQUINONES (CONCENTRATION 0.1%, SOLVENT 95% ETHYL ALCOHOL)

Compound	Positions on the Ring							Clearance,[a] Mm.
	1	2	3	4	5	6	7	8	
1,4-Naphthoquinone	O	H	H	O	H	H	H	H	5
2-Methyl-1,4-naphthoquinone	O	CH_3	H	O	H	H	H	H	17
2-Amino-1,4-naphthoquinone	O	NH_2	H	O	H	H	H	H	12
2-Chloro-1,4-naphthoquinone	O	Cl	H	O	H	H	H	H	12
2-Phenyl-1,4-naphthoquinone	O	C_6H_5	H	O	H	H	H	H	7
2-Isoheptyl-1,4-naphthoquinone	O	$(CH_2)_4C(CH_3)_2$	H	O	H	H	H	H	4
2-Sulfanilamino-1,4-naphthoquinone	O	$NHSH_2C_6H_4NH_2$	H	O	H	H	H	H	None
2,3-Dichloro-1,4-naphthoquinone	O	Cl	Cl	O	H	H	H	H	3
2-Isoamyl-3-hydroxy-1,4-naphthoquinone	O	$(CH_2)_2CH(CH_3)_2$	OH	O	H	H	H	H	6
M266[b]	O		OH	O	H	H	H	H	8.5
M1916[b]	O		OH	O	H	H	H	H	None
M2279[b]	O		OH	O	H	H	H	H	None
M2309[b]	O		OH	O	H	H	H	H	None
M2892[b]	O	$(CH_2)_8CH(CH_3)_2$	OH	O	H	H	H	H	3
2-Methyl-3-hydroxy-1,4-naphthoquinone (phthiocol)	O	CH_3	OH	O	H	H	H	H	5.5
2-Methyl-3-phytyl-1,4-naphthoquinone	O	CH_3	Phytyl	O	H	H	H	H	None
2-Amino-1,4-naphthoquinonimine hydrochloride	O	NH_2	H	NHHCl	H	H	H	H	20
5,8-Dihydroxy-1,4-naphthoquinone	O	H	H	O	OH	H	H	OH	8.8
4-Amino-1,2-naphthoquinone	O	O	H	NH_2	H	H	H	H	None
4-*p*-Sulfanilino-1,2-naphthoquinone	O	O	H		H	H	H	H	None

[a] Cleared zone of growth inhibition around solution cup in agar plate, measured in millimeters as radius segment.
[b] The designation of these compounds from Fieser, L. F., and co-workers, in press.

creased the activity, whereas chlorine substitutions apparently decreased its fungistatic effect under test conditions. Longer side chains, as the butyl or the tridecyl substitutions, either diminished or abolished the fungistatic activity of *p*-quinone.

Similar conditions prevailed with the twenty investigated compounds of the naphthoquinone series (Table II). A methyl group in the 2 position enhanced the fungistatic activity of 1,4-naphthoquinone approximately three and one-half times. An amino group or chlorine in the same position also augmented this effect but not as greatly as the methyl substitution. A phenyl group in the 2 position exhibited only a slight positive influence on the basic fungistatic value of 1,4-naphthoquinone.

The potentiating influence of the methyl-substituted 2 carbon seemed to be almost completely curtailed by the introduction of a hydroxyl group in the 3 position and abolished by a phytyl chain at the same location. The introduction of chlorine to the ring (2,3-dichloro-1,4-naphthoquinone) appeared to diminish the fungistatic activity of 1,4-naphthoquinone. (Unfortunately, the monochloro-substituted compound was not available to us.) This situation disagrees completely with that of the plant fungicides reported by Ter Horst (5), where the introduction of chlorine atoms exerts a positive influence on the demonstrable fungistatic activity.

Various other groupings on the 2 position of naphthoquinone do not contribute much information. However, it may be said that occupation of the 3 position by a hydroxyl group slightly increased the basic fungistatic activity of 1,4-naphthoquinone in one instance (M266), slightly decreased this activity in one instance (M2892 or N-decylonyl-3-hydroxy-1,4-naphthoquinone), and caused no appreciable change with two other compounds (2-isoamyl-3-hydroxy-1,4-naphthoquinone and 2-methyl-3-hydroxy-1,4-naphthoquinone). This is in contrast with the suggestion of Colwell and McCall (7) that naphthoquinones require a free 3 position to react with sulfhydryl compounds in enzyme systems essential to the life of the fungus.

Of considerable theoretical interest is the outstanding action of 2-amino-1,4-naphthoquinonimine hydrochloride, the fungistatic value of which surpassed those of all the other compounds tested with this method. The activity of the only 5,8-substituted compound in this series, 5,8-dihydroxy-1,4-naphthoquinone, was only slightly greater than that of 1,4-naphthoquinone itself. The two investigated orthoquinones, 1,2-naphthoquinone and 4-*p*-sulfanilino-1,2-naphthoquinone, exhibited no activity.

Of the entire group of twenty-four quinoline derivatives (Table III), only three compounds showed a fungistatic response at the concentration

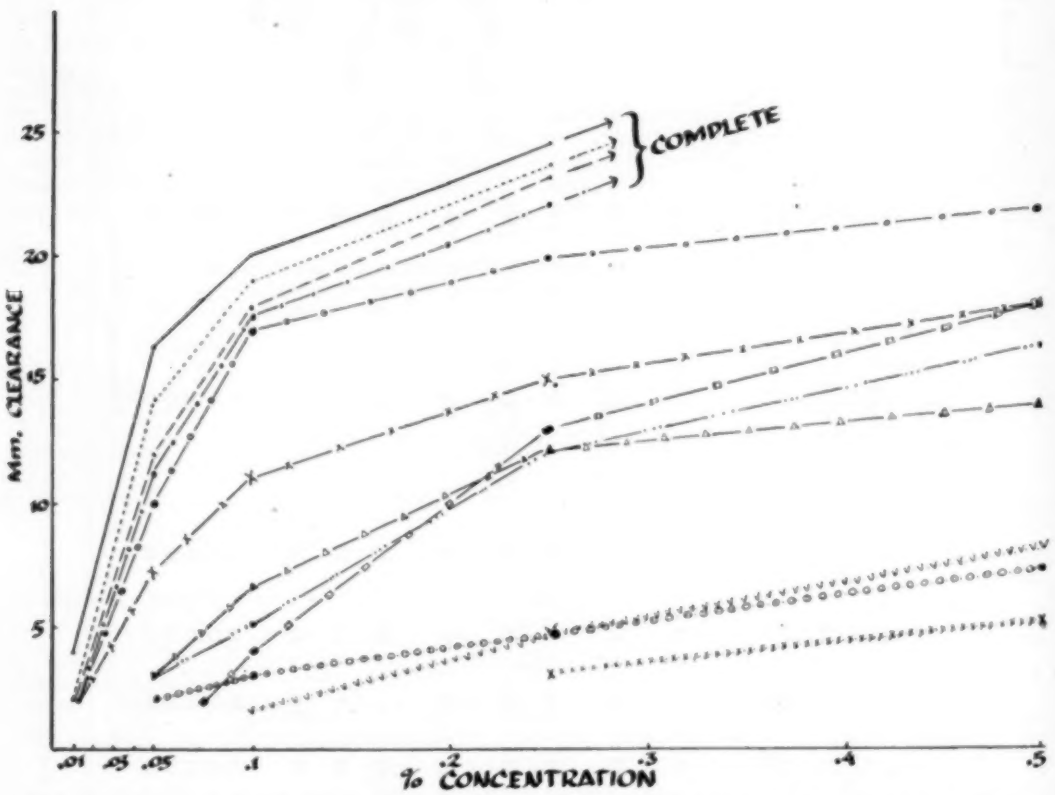


Fig. 1.—Dosage and fungistatic response curves of *T. mentagrophytes* (No. 640 Emmons) with increasing concentrations of various quinones and 8-hydroxyquinoline. ——, 2-amino-1,4-naphthoquinonimine HCl; ------, xyloquinone; – – – –, thymoquinone; ———, 8-hydroxyquinoline benzoate; o—o—o, 2-methyl-1,4-naphthoquinone; x—x—x, toluquinone; △—△—△, quinone; ·····, 1,4-naphthoquinone; □—□—□, toluhydroquinone; o·o·o·o·o, 2,3-dichloro-1,4-naphthoquinone; v·v·v·v·v·, 2,6-dichloroquinone; x·x·x·x·x·x·, hydroquinone.

TABLE III.—INFLUENCE OF VARIOUS SIDE GROUPS ON THE FUNGISTATIC ACTIVITIES OF 24 QUINOLINES (CONC. 0.1%, SOLVENT 95% ALCOHOL)

Compound	Clearance,[a] Mm.
8-Hydroxyquinoline	17.5
2,6-Dimethylquinoline	None
4,8-Dimethyl-2-hydroxyquinoline	None
3-Cyclohexyl-2,4-dihydroxyquinoline	None
7-Methoxy-4-hydroxyquinoline	None
7-Methoxy-2-methyl-4-hydroxyquinoline	None
3-Bromo-4-hydroxyquinoline	None
6-Methoxy-8-nitro-4-hydroxyquinoline	None
6-Methoxy-3(3-cyclobexylpropyl)-2,4-dihydroxyquinoline	None
6-Chloro-3(3-cyclohexylpropyl)-2,4-dihydroxyquinoline	None
3(3-Cyclohexylpropyl)-4-hydroxyquinoline	None
3(3-Cyclohexylpropyl)-2,4-dihydroxyquinoline	None
6-Dimethylamino-3(3-cyclohexylpropyl)-2,4-dihydroxyquinoline	None
5,6 (or 6,7-)-Dimethyl-4-hydroxyquinoline	None
6-Aminoquinoline	None
8-Aminoquinoline	None
6-Nitroquinoline	None
8-Nitroquinoline	None
5-Nitroso-8-hydroxyquinoline	None
5-Amino-8-hydroxyquinoline	None
5,7-Dibromo-8-hydroxyquinoline (dissolved in acetone)	3.0
8-Amino-6-methoxyquinoline	None
5-Chloro-7-iodo-8-hydroxyquinoline (dissolved in acetone)	3.0
quinine alkaloid	None

[a] Cleared zone of growth inhibition around solution cup in agar plate, measured in millimeters as radius segment.

possesses an almost specific fungistatic affinity for *Trichophyton mentagrophytes*. Similar findings have been previously described (12), using 8-hydroxyquinoline against a plant fungus and with a different test method. Naturally, the possibility exists that the other investigated quinolines (and quinones) might exhibit fungistatic activities at higher concentrations. The authors' present research is concerned with the mechanism involved in this fungistatic action of 8-hydroxyquinoline, especially as regards the possible inactivation of trace metals necessary for normal fungus growth.

Table IV reveals the wide range of fungistatic activity coefficients of twelve selected compounds. The coefficient of one compound, 2-amino-1,4-naphthoquinonimine hydrochloride, was as much as seventy-seven times higher than that of undecylenic acid, at present a popularly used antifungal agent, with a fungistatic activity coefficient of only 1 (1). Determination of fungistasis, using the two different techniques (Schamberg and Kolmer, Burlingame and Reddish), does not give completely parallel results (1). However, four compounds appeared to be outstanding under the conditions of both test methods, *p*-xyloquinone, *p*-thymoquinone, 8-hydroxyquinoline, and 2-methyl-1,4-naphthoquinone (menadione).

The high fungistatic activity of 2-amino-1,4-naphthoquinonimine hydrochloride, as demonstrated by the Burlingame and Reddish method, could not be duplicated when using the Schamberg and Kolmer technique. The compound is rather unstable and the procedure of heating the agar in which the compound is to be incorporated may facilitate a hydrolysis of the test substance (15). The low value displayed by *p*-toluquinone with this incorporation technique was rather surprising in comparison with its relatively high fungistatic activity coefficient with the agar cup-plate method. However, this compound exhibited a lower ceiling value in the dosage-response curves than would appear likely from its activity coefficient. 2,3-Dichloro-1,4-naphthoquinone exhibited considerable fungistasis with this (Schamberg and Kolmer) technique, yet had a low fungistatic activity coefficient. The problem of penetration through the agar medium due to the rather low solubility of this compound

TABLE IV.—FUNGISTATIC ACTIVITY OF TWELVE COMPOUNDS EXPRESSED IN TERMS OF ACTIVITY COEFFICIENTS AND MAXIMUM DILUTION FOR FUNGISTASIS

Compound	Maximum Dilution Effecting Fungistasis (Schamberg and Kolmer Method)	Fungistatic Activity Coefficients Derived From Dosage-Response Curves (Burlingame and Reddish Method)	
		% Conc. to Give 5-Mm. Clearance	Activity Coefficient
2-Amino-1,4-naphthoquinonimine HCl	1: 35,000	0.013	77.0
p-Xyloquinone	1:100,000	0.020	50.0
p-Thymoquinone	1: 70,000	0.0225	44.4
8-Hydroxyquinoline Benzoate	1: 75,000	0.025	40.0
2-Methyl-1,4-naphthoquinone (Menadione)	1: 60,000	0.0275	36.3
p-Toluquinone	1: 5,000	0.035	28.5
p-Benzoquinone	1: 4,000	0.0775	13.0
1,4-Naphthoquinone	1: 4,000	0.10	10.0
Toluhydroquinone	1: 1,000	0.1275	8.0
2,6-Dichloroquinone	1: 2,500	0.2775	3.6
2,3-Dichloro-1,4-naphthoquinone	1: 35,000	0.2825	3.5
Hydroquinone	1: 2,500	0.5	2.0

of 0.1%. Two of them, 5,7-dibromo-8-hydroxyquinoline and 5-chloro-7-iodo-8-hydroxyquinoline, were dissolved in acetone because of their insolubility in alcohol. The fungistatic activity of the third, 8-hydroxyquinoline, was outstanding, paralleling the most effective fungistatic compounds of the investigated quinone series. All of the substitutions tested, introduction of other hydroxy, methyl, methoxy, amino, nitroso, and nitro groups, and halogens, abolished the activity of the compound. It would seem that 8-hydroxyquinoline

TABLE V.—MAXIMUM NONFUNGICIDAL AND MINIMUM FUNGICIDAL CONCENTRATIONS OF TEN COMPOUNDS, USING A 10-DAY CULTURE OF *Trichophyton Mentagrophytes* ON SABOURAUD'S MALTOSE AGAR PLUS 10% SERUM

Compound	Maximum Nonfungicidal Conc.	Minimum Fungicidal Conc.
2-Amino-1,4-naphthoquinonimine HCl	0.075	0.1
p-Xyloquinone	0.075	0.1
p-Toluquinone	0.1	0.25
2-Methyl-1,4-naphthoquinone	0.1	0.25
p-Thymoquinone	0.1	0.25
1,4-Naphthoquinone	0.1	0.25
p-Quinone	0.5	0.75
8-Hydroxyquinoline Benzoate	0.5	0.75
Toluhydroquinone	5.0	6.0
Hydroquinone	15.0	..

might have been responsible for the discrepancy.

Any laboratory evaluation of an antifungal substance should include specific tests for the determination of its fungicidal potency. The twelve selected compounds were subjected to the authors' modified assay procedure for fungicidal compounds (2). Two compounds, 2,6-dichloroquinone and 2,3-dichloro-1,4-naphthoquinone, could not be evaluated due to their limited solubilities in alcohol. Hydroquinone was not fungicidal even in a 15% concentration and was therefore disregarded as a fungicide. The nine remaining substances showed various grades of fungicidal activity. 2-Amino-1,4-naphthoquinonimine hydrochloride and *p*-xyloquinone, the most active compounds of the group, were fungicidal in a concentration of 0.1%. This is twenty-five times higher than undecylenic acid, which was found to be fungicidal in a 2.5% concentration under the same test conditions. 8-Hydroxyquinoline, which exhibited excellent fungistatic properties, was fungicidal in a concentration of 0.75%.

The fungicidal activities indicated here are valid only when employing 95% alcohol as the solvent. Any decrease in the alcohol concentration necessitates an increase in the concentration of the test material, providing all of the other test conditions are identical.

SUMMARY

1. Eleven derivatives of benzoquinone, twenty derivatives of naphthoquinone, and twenty-four quinoline derivatives, dissolved in 95 per cent ethyl alcohol in a concentration of 0.1 per cent, were evaluated for their fungistatic activities.

2. Twelve selected compounds were subjected to more intensive laboratory investigation to determine their fungistatic activity coefficients and their fungicidal properties.

3. It could be shown that four compounds of this series, *p*-xyloquinone, *p*-thymoquinone, 2-methyl-1,4-naphthoquinone (menadione), and 8-hydroxyquinoline, possess outstanding antifungal qualities.

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CORRECTION

In the article entitled "Digitalis VII. The Effect of Various Alkalies on the Sensitivity of the Baljet Reaction for Digitoxin", THIS JOURNAL, 37, 297–301(1948) Table I, page 299, should read as follows:

TABLE I.—OPTICAL DENSITY × 100

Expt.	NaOH Conc., %	Picric Acid Conc., %	Time (in Minutes) After Addition of Reagent 5	10	20	30	40	50	60	70	80	90	120	150	180
1	0.25	0.5	25.0	30.5	35.0	36.5	37.4	37.5	37.1	37.0	37.1	37.1	37.0	36.6	36.5
2	0.5	0.5	37.5	39.5	39.0	38.9	39.0	38.9	38.4	38.7	38.0	38.0	38.0	37.5	37.1
3	0.5	0.25	35.7	39.6	40.1	40.1	40.2	40.0	39.4	39.4	39.5	39.0	38.3	37.6	36.5

Enhancement of Penicillin Activity *in vitro* by Vitamin K_5*,†

By ROBERTSON PRATT, JEAN DUFRENOY, and PETER P. T. SAH
with the laboratory assistance of VIRGINIA L. PICKERING

Vitamin K_5 (2-methyl-4-amino-1-naphthol hydrochloride) in concentrations from 0.1 to 10 mg./L. enhances the effectiveness of penicillin *in vitro* against *Staphylococcus aureus, Proteus vulgaris,* and *Escherichia coli*. Depending upon the test organism used, the zones of inhibition around cylinders containing a solution with 1 unit penicillin and 1 microgram K_5/ml. were as large as could be obtained around cylinders containing penicillin alone in concentrations ranging from 1.5 to 100 units/ml. Since the activity of combinations of vitamin K_5 and of penicillin in suitable concentrations is greater than would be expected from the sum of the activities of the two compounds acting separately, the effect appears to be one of synergism rather than merely the result of two antibacterial agents acting simultaneously but independently.

AN AGENT that is capable of increasing the effectiveness of penicillin is potentially a valuable adjunct to the clinicians *armamentarium*. Reports of the enhancement of the antibiotic activity of penicillin by various agents have been in the literature since 1944 when Cornman (1, 2) and Lewis (3) independently observed that partially purified penicillin exerted a selective lethal action *in vitro* against rat and mouse sarcoma cells whereas pure penicillin possessed no such activity. In 1945 Dunham and Rake (4) showed that partially purified penicillin reduced the motility of cells of *Treponema pallidum* and that crystalline penicillin did not do so. Independently, Smith (5) and de Ropp (6) reported retardation of seed germination and of root growth in solutions of crude or of partially purified penicillin. Among the impurities that have been isolated from amorphous penicillin and that might be expected to induce the observed results are indole acetic acid, phenylacetic acid, orthohydroxyphenylacetic acid, members of vitamin complexes, and other unidentified growth-regulating factors. These are discussed in a recent review (7). Recently Lecoq and Solomides (8) reported that only one-half as much penicillin or streptomycin was required to inhibit growth of *Escherichia coli* in nutrient broth containing one part of para-amino-benzoic acid in 150 parts of broth as in broth lacking PAB.

Turning to *in vivo* studies, Welch, Randall, and Price (9) reported the occurrence in a number of commercial lots of amorphous penicillin of an unidentified factor which, though devoid of protective activity itself, enhanced the protective action of penicillin against *Eberthella typhosa* in mice as much as 100 per cent in some cases, i.e., 2000 units of amorphous penicillin containing the factor or of crystalline penicillin with the factor added were as effective as 4000 units of crystalline penicillin alone. Pursuing that line of investigation, Hobby, Lenert, and Hyman (10) observed that impure penicillin was 3 to 5 times more effective than crystalline penicillin G in protecting mice against *Streptococcus hemolyticus*. Likewise, impure penicillin afforded greater protection against *Diplococcus pneumoniae* infections in mice. The factor reported by Welch, *et al.*, and Hobby, *et al.*, appeared not to be correlated with the amount of phenylacetic acid present or with degradation products of penicillin, although other observations have indicated that phenylacetic acid, sodium acetate, and perhaps even normal physiological saline may enhance the activity of crystalline benzyl penicillin under certain conditions (11). Hobby, *et al.* also observed that the chemotherapeutic activity of crystalline penicillin G may be enhanced by a number of dissimilar substances, among which are penicillin K and inactivated penicillinase.

It has been shown in this laboratory that appropriate trace amounts of cobalt, nonbacteriostatic *per se*, increase at least twofold the protective action of crystalline penicillin G against infection of mice by *E. typhosa* (12). The enhancement of penicillin action by cobalt differs from the enhancement induced by the factor reported by Welch, *et al.* (9), Hobby, *et al.* (10), and others (11) in that it is readily demonstrated *in vitro* (13) and in that the concentrations of cobalt required to produce the effect are extremely low.

The trend in the pharmaceutical industry and in medicine has ever been away from the use of unidentified or crude preparations and toward the goal of administration of definite amounts of known compounds. Thus, if the use of adjuvants to enhance or to potentiate the action of penicillin should some day be deemed clinically

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† This research was supported in part by a generous grant from the Cutter Laboratories, Berkeley, Calif.

practicable, it would seem desirable to offer the profession chemically defined compounds. The observations on cobalt (12, 13) represented such an advance. However, although the concentrations of cobalt that were used were well below the toxic level, some opposition to its use in clinical practice might be anticipated because of its potential toxicity in the event of misuse. Therefore, the discovery of other agents that are capable of enhancing penicillin activity but that are potentially less dangerous would be most welcome. The present paper reports the use of such a compound, i.e., vitamin K_5 (2-methyl-4-amino-1-naphthol hydrochloride), which is well known for its antihemorrhagic properties. Veldstra and Wiardi (14) reported the *LD* for oral administration in rats as being 0.7 Gm./Kg. However, they failed to state whether this figure represented the LD_{50} or LD_{100}. Sah and Anderson (unpublished data) found the LD_{50} for mice to be 750 mg./Kg. for intraperitoneal administration.

EXPERIMENTAL

Vitamin K_5 (2-methyl-4-amino-1-naphthol hydrochloride) was synthesized as previously described by one of us (15) with slight modifications to improve the yield.

Estimates of the antibacterial effectiveness of the penicillin, a preparation of crystalline sodium benzyl penicillin that assayed 1600 units/mg., were made by the cylinder-plate technique, using both the standard overnight assay and the rapid assay involving physical development (16). *Staphylococcus aureus* NRRL 313, *Proteus vulgaris* HX19, and a laboratory isolate identified as *Escherichia coli* were used as test organisms.

RESULTS

Vitamin K_5 Incorporated in Test Agar.—Incorporation of appropriate amounts of vitamin K_5 in the nutrient agar resulted in formation of larger zones of inhibition than occur normally around cylinders containing a given concentration of penicillin on assay plates seeded with *S. aureus*, with *P. vulgaris*, or with *E. coli*. The apparent enhancement of penicillin activity occurred whether the assay was performed by means of the standard procedure or by means of the rapid cylinder-plate technique described previously (16). The results are summarized in Figs. 1 to 3.

The enhancement was generally greater at the lower concentrations of penicillin, and was more pronounced on plates seeded with Gram-negative *E. coli* or *P. vulgaris* than on those seeded with *S. aureus*. For example, the maximum enhancement of activity against *S. aureus* was less than two-fold but a ten- to twenty-fold increase in activity was noted against *E. coli:* on five-hour assay plates with 1 mg. K_5/L. (1 p. p. m.) a solution with 0.5 unit penicillin/ml. produced zones equivalent to those produced by 5 units/ml. on the regular me-

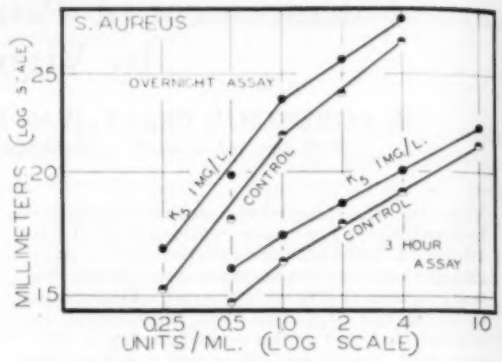

Fig. 1.—Diameter of inhibition zones formed around cylinders with different concentrations of penicillin tested against *Staphylococcus aureus* seeded on the standard medium and on the same medium containing vitamin K_5.

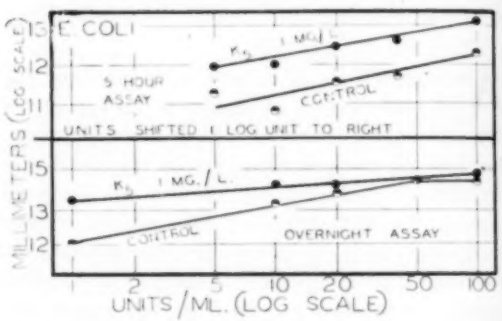

Fig. 2.—Diameter of inhibition zones formed around cylinders with different concentrations of penicillin tested against *Escherichia coli* seeded on the standard medium and on the same medium containing vitamin K_5.

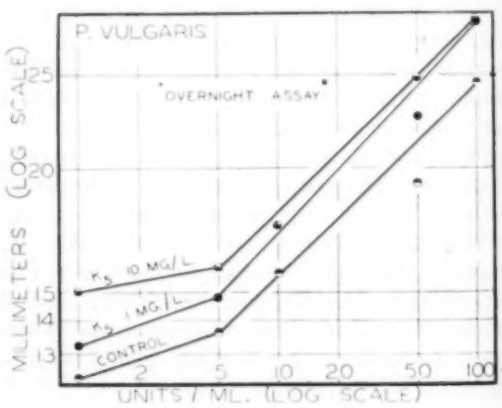

Fig. 3.—Diameter of inhibition zones formed around cylinders with different concentrations of penicillin tested against *Proteus vulgaris* seeded on the standard medium and on the same medium containing vitamin K_5.

dium, and in overnight assays 1 unit penicillin/ml. produced zones on K_5 medium equivalent to zones produced by 20 units penicillin/ml. on standard medium. The maximum increase in penicillin effectiveness against *P. vulgaris* was approximately eightfold.

Vitamin K_5 Dissolved in Solutions of Penicillin.—Vitamin K_5 and some of its analogs and related compounds are known to inhibit the growth and other metabolic activities of several species of bacteria, fungi, yeasts, and algae (17–22). Therefore, it seemed of interest to ascertain whether the effect noted above was actually due to enhancement of penicillin activity or whether it should be attributed merely to the additive effect of two antibacterial agents acting simultaneously.

Accordingly, solutions with different concentrations of vitamin K_5 and of penicillin were assayed by the standard cylinder-plate technique (sixteen hours' incubation and standard medium) using *S. aureus* and *E. coli* as the test organisms. The averaged results of this series of experiments plotted in Fig. 4 suggest that in appropriate concentrations vitamin K_5 and penicillin act synergistically, since the combined antibacterial action of the two agents was greater than would be expected from the sum of their independent actions. This was most pronounced in the case of the penicillin-resistant, Gram-negative *E. coli*. A solution containing 1 mg. K_5/L. (1 p. p. m.) produced zones of inhibition approximating in diameter the zones produced by a solution containing 0.5 unit penicillin/ml. But a preparation with 0.5 unit penicillin/ml. fortified with 1 mg. K_5/L. yielded inhibition zones larger than those produced by 10 units penicillin/ml. alone, and the zones produced around cylinders with 1 unit penicillin/ml. similarly fortified with K_5 were larger than those produced by 100 units penicillin/ml.

In the case of plates seeded with *S. aureus* the effect was not so pronounced. Vitamin K_5 alone in the penicylinders at a concentration of 1 mg./L. (1 p. p. m.) produced zones that averaged 12.5 ± 0.4 mm. in diameter. Such zones are considerably smaller than those produced by a penicillin solution containing 0.25 unit/ml. Addition of 1 mg. K_5/L. to solutions containing 0.5 or 1 unit penicillin/ml. gave zones equal to those produced by 0.75 and 1.4 units penicillin/ml., respectively.

The enhancement of penicillin activity by vitamin K_5 was noted with concentrations of the latter ranging from 0.1 mg. to 10 mg./L. but a concentration of 1 mg./L. gave maximum enhancement against *E. coli* and *S. aureus*. The optimum concentration of K_5 for increasing the effectiveness of penicillin against *P. vulgaris* was not determined but it appears to be greater than 1 p. p. m. (see Fig. 3).

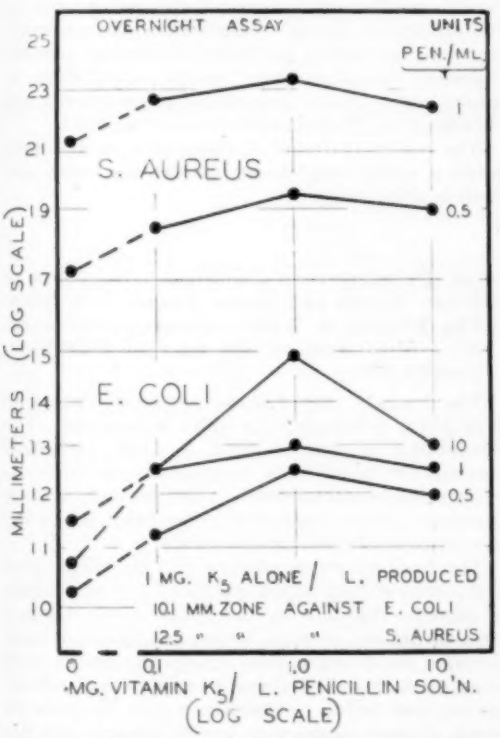

Fig. 4.—Diameters of inhibition zones formed around cylinders with different concentrations of penicillin and of vitamin K_5 tested against *Staphylococcus aureus* and against *Escherichia coli* seeded on the standard medium.

SUMMARY

A study was made of the effect of vitamin K_5 (2-methyl-4-amino-1-naphthol hydrochloride) in enhancing the activity of benzyl penicillin against *Staphylococcus aureus*, *Proteus vulgaris*, and *Escherichia coli*. Tests were performed by the cylinder-plate technique.

The magnitude of the enhancement depended upon the conditions of the experiment, the concentrations of K_5 and of penicillin, and the test organism. Under optimal conditions K_5 incorporated in the nutrient agar (1 mg. K_5/L.) increased the apparent activity of penicillin approximately twofold against *S. aureus*, eightfold against *P. vulgaris*, and twenty-fold against *E. coli*. Solutions containing 1 unit of penicillin and 1 microgram of K_5/ml. when placed in penicylinders on standard agar exerted activity against *S. aureus* equivalent approximately to the effect of 1.4 units penicillin/ml. alone. When *E. coli* was the test organism the same solution produced an effect greater than that of 100 units penicillin/ml.

The optimum concentration of K_5 for enhancing the effectiveness of benzyl penicillin is about 1 microgram/ml. of penicillin solution for *S. aureus* and *E. coli*, although the enhancing effect obtains over the range 0.1 to 10 micrograms/ml.

The activity of combinations of vitamin K_5 and of penicillin in suitable concentrations is greater than would be expected from the sum of the activities of the two agents acting separately. Therefore, it is concluded that vitamin K_5 and penicillin act synergistically.

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Book Reviews

Toxicology and Hygiene of Industrial Solvents. Edited by K. B. LEHMAN and F. FLURY. Translated from the German by ELEANOR KING and HENRY F. SMYTH, JR. The Williams & Wilkins Company, Baltimore, 1943. xiv + 378 pp. 14.5 x 23 cm. Price $5.

Perhaps the most common chemical industrial hazard arises from the misuse of industrial solvents. The authors have brought together the factors of interest in industrial toxicology in this field.

The first portion of this book is concerned with the chemistry (including analysis), the preparation, and the use of solvents. The general toxicology of solvents is then considered. This is followed by the main section of the book which deals with the individual solvents. Hydrocarbons, chlorinated hydrocarbons, alcohols, esters, ethers, aldehydes, ketones, terpenes, and miscellaneous solvents are considered here. A short chapter on skin injuries follows and this in turn is followed by sections on preventive measures and official German regulations.

At first glance this book appears to be of limited pharmaceutical interest. Further study shows considerable data on glycols, ethyl acetate, dibutylphthallate, dimethylphthallate, ether, chloroform, carbon tetrachloride, and many other compounds used in pharmacy. In addition the discussions of experimental toxicology may serve as points of departure for other studies by the research worker.

Chromatography. By H. G. CASSIDY, N. APPLEZWEIG, S. CLAESSON, V. R. DEITZ, B. J. MAIN, A. J. P. MOORE, R. L. PECK, W. A. SCHROEDER, L. SHEDLOVSKY, W. H. STEIN, H. C. THOMAS, and L. ZECHMEISTER. Annals of the New York Academy of Sciences, February 10, 1948. 186 pp. 15 x 23.5 cm.

This booklet represents the report of a conference on chromatography held at the New York Academy of Sciences.

Dr. Zechmeister, one of the early founders of this interesting method of analysis, wrote the introductory chapter on the history, scope, and basic methods. Frontal analysis and experiments in systematic quantitative methods follow. After a section on the applications of chromatography to the problem of spacial configurations and to streptomycin, methods of partition chromatography are discussed including their specific application to amino acid analysis. Ion exchange adsorbents and classifications of solid adsorbents are included.

The review of recent developments in this field forms a useful supplement to reference books and texts in chromatography.

Blood Derivatives and Substitutes. By CHARLES STANLEY WHITE and JACOB JOSEPH WEINSTEIN. The Williams & Wilkins Company, Baltimore, Md., 1947. xviii + 484 pp. 15 x 22.5 cm. Price $7.50.

The dramatic role of blood and blood plasma in World War II is fresh in the mind of everyone. The availability of human blood in large quantities through the blood bank programs led to many developments in fractionation. The research on these fractions, which is explained in this book, led to the development of many unique uses.

This book covers, in addition to a historical count and a brief section on the chemistry and physiology of plasma, chapters on the preparation of liquid citrated plasma, storage, drying of plasma, administration, and fractionation. A chapter dealing with plasma substitutes covers such substances as gelatin, pectin, and acacia. There are also chapters on shock, clinical results with human plasma, and untoward reactions with plasma.

The addition of about 200 graphs, charts, and pictures aids in making this an authoritative work in this important field.